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The American Phytopathological Society

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An International Journal
Official Organ of The American Phytopathological Society

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PHYTOPATHOLOGY

VOLUME 21 NUMBER 6

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THE METHODS OF CLASSIFICATION OF PLANT VIRUSES, AND AN ATTEMPT TO CLASSIFY AND NAME POTATO VIROSES[1]

H. M. QUANJER[2]

INTRODUCTION

For the viroses of no other plant is the necessity of a logical and internationally understandable classification more urgent than for those of the potato plant. This may be illustrated by the following example:

The common mosaic of the potato variety Bliss Triumph, called "rugose mosaic" in some papers from the Agricultural Experiment Station of the University of Wisconsin (among others, 34), is, according to Johnson (41), not identical with the rugose mosaic of Schultz and Folsom but with their "crinkle mosaic." But, according to Johnson, it is not identical with the "crinkle" of Murphy and McKay nor with that of Quanjer. It, however, resembles more closely the simple or common mosaic of these European authors.

In order to avoid future difficulties of this kind, Johnson attempted to identify and differentiate potato viruses by the determination *in vitro* of certain physical and chemical characteristics which he called the "properties" of the viruses concerned. According to him, the most reliable diagnostic features of the "property" type are the thermal death-point, the longevity *in vitro*, the effect of dilution, and the influence of certain chemicals.

[1] A preliminary sketch of this paper has been presented to the Fifth Botanical Congress, held at Cambridge, August, 1930.

[2] Virus-free material was grown for the writer by Dr. Oortwijn Botjes. Material was sent from abroad by Dr. Cummings, Dr. K. H. Fernow, Dr. Paul A. Murphy, and Dr. E. S. Schultz. In executing the intervarietal transmission experiments, on which are based the results presented, the writer was assisted, in 1927, by Ir. E. Biewinga; in 1928, by Dr. V. Likhité; in 1929, by Ir. C. C. Klijnhout; and in 1930, by Dr. J. H. H. van der Meer. A part of the microscopical work was done by Mr. A. Ovinge. Suggestions or criticisms in the presentation of the results and help in the editing of the English text of this paper were kindly given by Dr. J. Henderson Smith, Dr. Lee M. Hutchins, Dr. James Johnson, Dr. Paul A. Murphy, and Dr. Geo. H. Pethybridge, with whom the writer discussed the subject during and after the Fifth International Botanical Congress. The photographs of figure 4 were made by Ir. K. Leendertz. The writer wishes to acknowledge the valuable suggestions and help of all persons mentioned.

While the determination of these properties offers great possibilities especially for the knowledge of the nature of the viruses concerned and is indeed helpful in clearing up some of the existing confusion, it cannot be expected to give much information on the properties of the viruses which are of primary interest to us, *i.e.*, their properties as pathogenes. It may also be noted in Johnson's work that the same descriptive names that he criticises and that have presented so many difficulties for international as well as for local use—"crinkle mosaic," "rugose mosaic," "leaf-rolling mosaic," and "mild mosaic," for certain diseases; and "wrinkling," "ruffling," "curl," "roll," "stem necrosis," "rugosity," and "streaking" for certain symptoms apparently are indispensable to his report.

The writer, in order to corroborate Johnson's results, has tried to approach the problem from the pathological side. It is evident that the value of the determination of physical and chemical characteristics of the viruses concerned must increase when, at the same time, their properties as pathogenes are elucidated. To this purpose it seemed desirable, first, to give a critical survey of all the methods thus far in use for the identification, differentiation, and classification of viruses and then to choose such methods as appeared to be most appropriate for elucidating the pathological side of the potato-virose problem.

Our knowledge of the virus disease of plants has been gathered by the following methods:

A. Indirect methods (pathological methods according to Quanjer).
   1. Symptomatology.
   2. Morbid anatomy and physiology of the hosts.
   3. Determination of the host range.
   4. Determination of the modes of transmission and of the relation between vectors and viruses.
   5. Determination of the effect of environment on the diseased hosts.

B. Method not yet classifiable as direct or indirect.
   6. Cytology (x-bodies).

C. Direct methods (property methods according to Johnson).
   7. Cultivation of the viruses and determination of their physical and chemical characteristics.

It is not the writer's intention to include in the present paper a survey of the entire literature, nor does he attempt more in the first seven chapters than to give an idea of what already has been accomplished through the use of the above methods and to indicate some additional progress that may be expected to be attained through the further application of these methods to the identification, differentiation, and classification of plant viruses.

In the eighth chapter an attempt will be made to present a classification and nomenclature of some potato viroses on a pathological basis.

CHAPTER 1.

SYMPTOMATOLOGY

That symptomatology plays an important rôle in all studies on virus diseases is evident when we try to determine what virus diseases are. The feature that induces us to combine such different phenomena as infectious chlorosis of abutilon (51), (6), tobacco mosaic (53), stipple streak of the potato (2), peach rosette (81), and alloiophylly of the anemone (43) is not the filterability of the causal agents; neither is it their transmissibility by insect vectors; nor the occurrence of easily visible, amoeboid, intracellular inclusions.

The most generally combining features are the infectiousness, the noncultivability of the viruses *in vitro*, the rapidity with which the viruses are taken up by the living cells once they are introduced into wounds made by insects or other agents, the typical way in which the viruses are spread throughout the plant, and, last but not least, the type of symptoms.

The symptoms differ entirely from those caused by bacteria and fungi, which, as a rule, are much more localized. It is true that from the point of inoculation certain bacteria and fungi may penetrate plant tissues without causing conspicuous injury as the infection progresses, but, upon arriving at a favorable location distant from the point of entry, they may cause local symptoms. When, however, healthy-looking sprouts are cut off from the diseased part of the plant and caused to root in soil or are grafted on a healthy stock, it is, as a rule, possible to grow them free from the rather localized pathogene. In the case of grafting, it is not easy for fungi or bacteria to grow through the uniting callus from scion to stock. In the case of viroses, however, it is almost or entirely impossible to get rid of the disease by cutting off tops or buds and growing them in soil or grafting them on healthy stocks. The virus passes easily from the scion to the stock.

In identification and differentiation, also, symptomatology plays a prominent rôle, since the presence of a certain virus can be determined only by the symptoms it produces after its inoculation. But it is not yet possible to develop a general system of classification on the basis of symptoms. That a special group of "infectious chloroses," for example, may be formed on a basis of resemblance of the group members to the noninfectious chloroses (30, Chapter 12, a. o.) is open to criticism. The fact that the virus, which, in *Abutilon thompsonii*, causes an ornamental variegation, has a necrotic effect on *A. indicum* proves the relation of this group to the so-called mosaics.

That the classification into "mosaics" and "yellows" (18) is also premature will be evident when we think of the great number of viroses, which, according to their clinical picture, could not be grouped under either of these headings: *e.g.*, Klebahn's "alloiophylly" of the anemone and other diseases for which the term alloiophylly seems apt, such as "reversion" or "nettle head" of the black currant (49), the "Quercina type" of *Datura stramonium* (11), the extreme dwarfing of spindling sprout (23) or its reverse, the "giant hill," in potatoes. Besides, the most classical representative of the mosaic diseases, caused by Johnson's tobacco virus No. 1, after being transmitted to *Capsicum annuum*, produces a chlorotic condition without mottling, *i.e.*, a yellows (34). Other critical remarks on the grouping into mosaics and yellows will be made in the following chapter. In spite of these criticisms, a classification into (a) "infectious chloroses," (b) "mosaics and related diseases, such as crinkles, streaks, and topnecrosis," (c) "yellows, leaf-roll, and curly top," and (d) "alloiophyllies" has been provisionally adopted by the writer for teaching purposes.

It must be conceded that one often feels uncertain in deciding on the virus nature of a disease when symptomatology is not corroborated by infection experiments, especially when the diseases in question are not systemic. Single or concentric chlorotic circles or lines on the leaves alternating with normal green tissue, and suggestive of the Liesegang phenomenon, are considered as symptoms of virosis. Examples are a mosaic of pea and one of peony (46) and a tobacco ring spot, all three occurring in the United States, and a disease occurring in the Scotch potato variety Arran Consul and in the Dutch variety Maaike and called "yellow barred" or "chevron marking" by Murphy. This last disease is systemic and graft-transmissible. Priode's tobacco ring-spot virus causes local symptoms in the mature leaf tissue around the point of inoculation, the symptoms usually becoming systemic afterwards; but, "A few instances were observed where systemic infection failed to follow infection" (65). One is inclined to attribute a disease to a virus origin when, as in the case of the concentric-ring necrosis of the potato tuber, characterized by more or less concentric necrotic circles around a lenticel (Fig. 8, C), it cannot be ascribed to a cultivable organism (3). But, in this disease we should then have an example of a virus pathogene entering a tuber from the soil and being restricted to the invaded tuber only, without being subsequently transported through the plant to the new tubers (67).

The writer is strengthened in the view that this disease is of virus nature by the work of Atanasoff (3), who grafted naturally infected tubers to newly dug healthy tubers and observed its development in the healthy potato in a most typical form.

Furthermore, other viroses are known which infect plants from the soil (56). A well-defined example of the restriction of a graft-transmissible disease to one organ of a plant is further given by Hutchins (37) in his work on the phony disease of the peach. He states, in part:

"At this time it became evident that the phony disease is contagious. Inoculations with the juice expressed from the roots or other parts of phony trees have not communicated the disease under the conditions of our experiments. It has thus far been artificially communicated only by means of grafting a phony root to the root system of the normal tree. This behavior places the disease in the peach yellows group, and the infective principle is now regarded as a virus. However, the phony disease differs from peach yellows in that the virus of phony disease has been found only in the roots of diseased trees and does not appear to enter the shoots above the soil line, while the peach yellows virus invades both shoot and root. This explains why the phony disease is not communicated when buds, scions or seeds from diseased trees are employed."

We have to acknowledge the enormous amount of information gained by the method of symptomatology on the viroses of special families. As examples may be mentioned Storey's work on mosaic and streak of sugar cane (87, 88, 89, 90), the studies of Bennett (8) and others on raspberry and of Plakidas (63, 64) on strawberry viroses, the work of Johnson (40) and of Valleau and Johnson (96) on the viroses of tobacco, of Bewley and Corbett (9) and others on those of tomato, and the work of Murphy and McKay (58) and of Schultz and Folsom (77, 78) on potato viroses.

CHAPTER 2.

MORBID ANATOMY AND PHYSIOLOGY OF THE HOST

Many viruses travel through the plant from inoculated leaves to growing points, young developing sprouts, storage places for reserve food, or the vegetative offspring, and sometimes to the sexual offspring, without leaving a trace of their path in the xylem, the intercellular spaces, or the walls of the parenchymatic tissues, as fungi and bacteria generally do. This suggested to several workers that the transport of the viruses and of the products of photosynthesis takes place along the same route. Beijerinck (7) was the first to consider the phloem tissues as the channels of transport of the contagium of tobacco mosaic. Baur (6), by girdling experiments, showed that the infectious agent causing chlorosis in Abutilon travelled down through the cortex or bast. Valeton (95), in his investigation of the sereh disease, found gummosis in the phloem but also in the xylem vessels of the sugar cane. Quanjer discovered phloem necrosis in an early study of potato leaf roll (66).

Later, the observations, seeming to support the view that the phloem and adjacent tissues are related to the distribution of viruses in the plant,

became more and more numerous. The galls of the Fiji disease are formed in the phloem (44). Absence of a fibrous sheath and appositional growth at the expense of the phloem strands has been found in Musa attacked by bunchy top (52) and clearing of the veins in *Musa textilis* attacked by a similar disease (60). Atrophy of the phloem vessels is seen in Morus suffering from dwarf (91). Clearing of the veins in the young leaves and phloem necrosis in the whole plant are characteristics of curly top of sugar beets (85). A disturbance resembling phloem necrosis occurs in advanced stages of the American (not the European) beet mosaic (72), (13). Necrosis in the phloem and pericycle is characteristic for curl of Rubus (70) and degeneration in the pericycle for xanthosis of Fragaria (63). Atrophy of xylem and phloem has been observed in *arricciamento* of Vitis (62). Clearing of the veins is the first symptom of some mosaic diseases of Solanaceae (73), (74), (82), and (84). Phloem necrosis occurs in the so-called "zeefvatenziekte" of Liberia coffee (86), the virus nature of which, however, is doubtful. Clearing of the veins also is observed as an early symptom of aster yellows (45).

Perhaps disturbance in the phloem would have been found in more cases had not this side of the problem been neglected by some investigators. Thung (93) proved by a very complete physiological comparison of healthy and of leaf-rolling potato plants that in the latter the accumulation of starch in the leaves can be attributed to neither a deficiency in the respiration nor in the diastatic activity of the mesophyll but only to disturbed translocation. This, in turn, must be a consequence of the pathological condition of the sieve tubes, a condition which ends in phloem necrosis. Accumulation of carbohydrates occurs, not only in potato leaf roll and in the sereh disease of sugar cane (99), mulberry dwarf (91), curly top of sugar beet (16), advanced stages of the American mosaic of sugar beet (72), curl of raspberry (21), *arricciamento* (curl) of Vitis (62), and aster yellows (21), in which at least some abnormalities in the phloem have been found, but also in the spike disease of Santalum (17), the spinach blight (94), peach yellows (10), leaf roll of Vicia (14), and the western or yellow blight of tomato (79), although the five latter diseases seem not to have been sufficiently investigated from a histological standpoint.

Further, in the case of some of the diseases in which abnormalities in the phloem have been found, *e.g.*, Fiji disease of sugar cane, bunchy top of *Musa* sp., xanthosis of Fragaria, and some diseases of the Solanaceae in which clearing of the veins has been found as an early symptom, we either have no knowledge or are insufficiently informed regarding the behavior of carbohydrates and other elaborated food in the leaves.

The histological investigation of different mosaics usually reveals a reduction in length of the palisade cells, a discoloration and reduction in

size of the chloroplasts, and a decrease in the amount of intercellular space in the lighter areas of the leaf. The mosaics are not so readily discernible by histological characters as they are by the pattern of outward symptoms. This seems to be the reason why Hoggan (34) concludes:

"It has not been found possible, as was originally hoped, to establish, to any great extent, any means of classification of viruses on the basis of the microscopical features of the diseased tissues!"

The writer will subsequently show in this paper that, at least for the viroses of the potato, more encouraging results have been reached by the histological method.

From the insufficiency of our knowledge of correlations between histological and physiological phenomena in many viroses it may be concluded that it would be premature to attach a too general significance to the following conclusion of Cook (18):

"These studies indicate that we may have two types of disease; the one generally represented by the various mosaics in which the photosynthetic activities are greatly reduced. The second which is represented by 'peach yellow' and 'little peach,' in which starch is formed in the leaves but not transported to other parts of the plant, the result being that the leaves become hard and leathery."

The same may be said of Dunlap's conclusion (21):

"The mosaic diseases were found to be accompanied by an increase of total nitrogen and by a decrease of total carbohydrates. The yellows diseases were accompanied by a decrease in nitrogen and by an increase in carbohydrate materials, as compared with the amounts of these constituents in normal tissue."

Cook's conclusions have value only for the diseases mentioned by him: sugar-cane mosaic, peach yellows, and little peach.

Those of Dunlap hold good only for tobacco, tomato, pokeweed, cucumber, and raspberry suffering from mosaic and for peach and aster suffering from yellows. A general classification based on their results would be too broad to be of real value.

It should not be inferred from what has been said above that the writer regards the movement through the phloem as the only mode of transport of viruses through the plant. It is thought to be responsible only for the spread over long distances; on the rate of this spread several observations have been made, *e.g.*, by Doolittle (19), who found that the cucumber-mosaic virus spreads through the vigorously growing cucumber plants from the point of inoculation and is present throughout the leaves and stems in four days, and by others whose observations are reviewed by Henderson Smith (32). At least some of the plant viruses can pass

through tissues other than the phloem. For example, tobacco virus No. 1 readily enters the leaves through broken trichomes, is immediately taken up by the living cells, and cannot be removed by washing (36). These facts point to the possibilities that there may be a close association between the virus and the protoplasm and that translocation of the virus may take place through the intercellular protoplasmic connections which are known to extend into and between the cells of the sieve tubes (75). If it is true that these "plasmodesms" form a continuous system throughout the plant, the above hypothesis would give an explanation of the peculiar way in which infection occurs and in which the viruses are spread through the infected plants. This hypothesis will be considered again in the chapter on cytology.

That the method of histological investigation sometimes may reveal mistakes made by a too superficial observation of symptoms or by an indiscriminate use of names will be shown in the last chapter. By this method (histological) it has been possible for the writer to confirm the difference between crinkle mosaic and rugose mosaic as made by Schultz and Folsom (77, 78) through the method of symptomatology and by Johnson (41) through the physical method and to divide into distinct groups some virus diseases collectively known as "streak."

It may be concluded from this rapid survey that much work is left to be done in the domain of morbid anatomy and physiology. Little or no attention has been paid to what happens in the plant during the incubation period; "clearing of the veins" precedes in some diseases the other symptoms, but that a more definite morphological and physiological description of this remarkable phenomenon seems not to be given is rather unsatisfactory.

CHAPTER 3.

DETERMINATION OF THE HOST RANGE

The host range of some plant viruses is very extended, *e.g.*, that of curly top, aster yellows, and cucumber mosaic (80), (45), (20), respectively. Some experiments, however, tending to prove the polyphagous nature of mosaic are open to criticism. Elmer (22) obtained mosaic transmission among species belonging to 15 different families of plants. That a mosaic virus can, as a consequence of transmission by artificial means, be forced to multiply in a given host and to produce mottling does not, however, prove that the mosaics occurring in nature are identical. Van der Meulen (97), employing natural vectors in attempts to determine the host ranges of mosaics occurring in plants belonging to different families, came to the conclusion that the mosaics are as a rule specialized. With the extension of

such experiments over a much wider range of plants, it may possibly be revealed that certain known viruses and supposedly different ones are, in fact, identical or are the same in regard to their causal relationship with abnormalities not yet recognized as viroses.

That the method of transmission in such experiments is of primary importance has been shown by Mogendorff (57). His results with the use of sap for the artificial transmission of cucumber mosaic to tomato were inconsistent and the percentage of infection was variable. Occasionally symptoms of "fern leaf" appeared, but they were not very typical. In most cases stunting and chlorosis were produced, sometimes accompanied by a faint mottling. Not until Hoggan (35) succeeded in transmitting cucumber mosaic from tobacco to tomato with the peach aphid did Mogendorff find that by means of this aphid, instead of using sap, he could transfer cucumber mosaic via *Nicotiana rustica* to tomato and obtain a high percentage of infection, with nearly all of the infected plants showing marked fern leaf.

The host-range method has been especially valuable for the discovery of "carriers," or plants in which a virose remains "latent." Such carriers have been detected in the genera Saccharum, Humulus, Beta, Abutilon, Phaseolus, Nicotiana, Physalis, and Solanum.

The value of differential hosts for identification work is limited by the fact that the pathogenicity of a virus may be decreased or increased by some of them. Carsner (15) and Lackey (47, 48) found not only that some weeds are difficult to infect with the curly-top virus but that the virus is "attenuated" by passage through them. The writer observed that a virulent acropetal necrosis of the potato was attenuated by passage through a seedling which behaved as carrier.

An example of what may be called increased virulence has been described by Johnson (39). The percentage of infection of a virus occurring in apparently healthy American potatoes was increased, the incubation period shortened, and the host symptoms accentuated, through successive cotton-needle transfers through tobacco, after which this virus was even capable of causing a serious necrotic disease when inoculated back into the variety of potato from which it was first extracted. Smith (82, 84) described a case of what seems to be the increase in virulence of a potato-mosaic virus. This virus, by needle-inoculation into tobacco, was induced to cause a ring spot in the latter plant and when brought back to potato, from which it was originally extracted, was induced to cause an intensified and killing mosaic. The possibility, however, may be considered that the mosaic potato plants of Smith contained two viruses. This consideration is based on the following facts. When scions of apparently healthy Zee-

land Blue potato plants are grafted on either of the varieties Duke of York or Green Mountain acropetal necrosis develops in the latter. When, however, parts of apparently healthy Duke of York or Green Mountain (which are carriers of the top-necrosis virus described in the last chapter of this paper) are grafted onto apparently healthy Zeeland Blue potato plants a mosaic develops in the grafted plants. It is clear that the grafted plants of this variety contain two viruses but show only mosaic. The method of differential hosts is, in some cases, apt for the analysis of such a virus complex. From the Zeeland Blue described above, virus causing acropetal necrosis can be brought into evidence by grafting on one of the potato varieties which is a regular carrier for top necrosis, and the topnecrosis can be made visible by grafting on one of the varieties in which the acropetal necrosis remains latent.

CHAPTER 4.

DETERMINATION OF THE MODES OF TRANSMISSION AND OF THE RELATION BETWEEN VECTORS AND VIRUSES

It is a remarkable fact and suggestive for further investigation that, as far as our knowledge goes, the groups of virus diseases mentioned in the first chapter are specialized in their modes of transmission. The diseases of group (a), infectious chloroses, are transmissible by grafting only. Those of group (b), mosaics and related diseases, are transmissible by grafting, as well as by sap and by insects. Those of group (c), yellows, etc., are, as a rule, transmissible only by grafting and by insects, whereas the modes of transmission of the members of group (d), alloiophyllies, are insufficiently known.

Some exceptions may be mentioned in group (b), mosaics. Neither the American nor the European sugar-beet mosaic seems to be easily transmissible with juice (72), (13). As exceptions in group (c), may be mentioned the following: "peach yellows" and "sandal spike," neither of which has thus far been possible to transmit by insects. Without any doubt, a more detailed study will reveal a greater number of exceptions and it may be possible also that group (a), infectious chloroses, will lose its isolated place.

It is clear that viroses transmissible by juice are separable from those transmissible solely by grafting or by insects and that both groups are separable from those transmissible solely by grafting. When a host contains two viruses, each of which has a strict relation to a different insect, separation is possible. An insect then may be used to isolate a virus from a juice- or graft-transmissible complex.

Severin (79) worked with tomatoes, which were suffering from both mosaic and yellows. In the tops of the shoots only the mosaic was visible.

He cut off these tops and placed them with the basal part in water and protected them by insect-gauze from the attacks of insects. Then virus-free males of *Eutettix tenellus* were allowed to feed on these shoots and were afterwards transmitted to healthy sugar beets. The latter were infected by the insect—which behavior gave evidence of the fact not only that the curly-top virus—which by previous experiments had appeared to be identical with the tomato-yellow virus—was present in the tops of the original tomatoes, but also that it is possible to use an insect adapted to a certain virus as a means of isolating this virus from a complex.

It is not necessary to dwell here on the relations between insects and viruses, which have been excellently surveyed in Kunkel's conspectus of virus diseases of plants (46). Elze (23) has used these relations as a means of classification. His recent work on this subject is presented in a paper in the current number of this journal.

Elze also showed that it would be a mistake, made by some investigators, to suspect insects that have been found in large quantities on a certain host to be the vectors of a virose attacking this host. Although leaf hoppers can often be found in great numbers on potato plants, they have no significance in the spread of leaf roll. Some spring migrants of *Myzus persicae* are sufficient to spread this disease in the beginning of the summer. They visit several plants in succession, depositing some young on each plant. Although, on the isle of Java, *Aphis sacchari* is much more common on the sugar cane than *A. maydis*, only the latter is a vector of sugar-cane mosaic.

This chapter may be concluded with the discussion of another example of what possibly may be regarded as the selection by the aphid *Myzus persicae* of one virus from a virus complex. Smith, in his experiments mentioned in the previous chapter, inoculated healthy tobacco with sap from a mosaic potato in two different ways, *i.e.*, by needle and by aphid. In the former case he obtained a ring spot, and in the latter a characteristic clearing of the veins developed as the first symptom on the young leaves, followed by the appearance of dark green lines along the veins, a phenomenon resembling what is called "vein banding" by Valleau and Johnson (96). Apart from increase in virulence, each of these diseases kept its own character in successive transfers through tobacco or back to potato, the former producing always a necrotic and lethal effect. In his first paper on this subject, Smith (82) believed that the mosaic potato contained only one virus. In his second paper, he concludes:

"It would appear that the aphis does not transmit some element of the virus which is concerned with the production of the more necrotic and lethal symptoms."

A third assumption would be that his original potato plant contained two viruses and that only one of these was transmitted by the aphis, whereas both are adapted for mechanical transfer.

The mosaic Zeeland Blue, mentioned in the end of the third chapter, shows a marked analogy with the mosaic potato plant experimented with by Smith; its virus, causing acropetal necrosis, is easily transferable through *Myzus persicae*, whereas thus far it seems impossible to transmit the top-necrosis virus by the aphid.

CHAPTER 5.

DETERMINATION OF THE EFFECT OF ENVIRONMENT ON THE DISEASED HOSTS

One general feature seems to hold good for all virus diseases, namely, that infection is independent of the presence of moisture. This may be attributed to the fact that the viruses are immediately taken up by the protoplasts as soon as insects or other agencies introduce them into the cells.

It is a well-known fact that the symptoms of some viroses do not develop at low temperature, *e.g.*, acropetal necrosis of potato (2) and tobacco mosaic in potato (12), while those of others are masked by high temperatures, *e.g.*, some potato mosaics (29). Top necrosis resembles the description of yellow dwarf made by Barrus and Chupp (5), but the fact that the latter disease does not show up in the cool summer climate of Holland proves that they are not identical.

Also, the manifestation and type of symptoms may be affected to some extent by external conditions, as was shown by Henderson Smith (31) in the case of a potato mosaic in tomato, where temperatures above 70° F. markedly reduced necrotic spotting and favored mottling.

Since the thermal conditions in the interior of a plant are to a certain extent independent of environment, such phenomena cannot be looked upon as due to the action of temperature on the virus; they must be dominantly due to its influence on the host.

Smith observed that temperatures above 80° F. masked the symptoms of the intensified potato mosaic, which, in his previously mentioned experiments, were obtained in potato after a transfer through tobacco and that the same temperatures did not affect the ring spot, *i.e.*, the form the disease takes in needle-inoculated tobacco. This would appear to be an example of the common experience that optimum conditions for the plant are optimum conditions for the appearance of symptoms. In looking over the details of Smith's experiments, one finds that not only is the needle-introduced ring spot in tobacco favored by high temperature but that the

aphis-introduced vein banding is likewise favored. This fact may have led Smith to his interpretation that one virus is responsible for both phenomena. If Smith is correct in this, we would have here an example of the influence of the means of transfer on the symptoms, showing some analogy with the result arrived at by Mogendorff and treated in the chapter on the determination of the host range. It is clear, however, that the question whether the mosaic plants from which his experiments were started contained one or two viruses cannot be solved by environmental studies and that we may anticipate that new experiments with differential hosts or differential treatment with physical or chemical agencies will throw further light on this problem.

CHAPTER 6.

CYTOLOGY

A survey of what has been learned from cytological investigations about the amoeboid, vacuolate intracellular inclusions, commonly called x-bodies, has been given (1) by Kunkel (46) and Likhité (50), who, along with Goldstein (27, 28) and others, consider these bodies to be stages in the life cycles of virus parasites, and (2) by Hoggan (34) and Henderson Smith (33), who, as did Iwanowski (38), the discoverer of the bodies, and as do most workers, incline to the view that these bodies are to be interpreted as products of the reaction of the infected cell to the presence of the virus.

It would be to no purpose to repeat here the arguments of the two groups; however, one remark may be made in favor of the latter. When we see how readily in irritated cells solids are precipitated and condensed on the starch grains, for example, the conception that the x-bodies may be inanimate by-products of the protoplast seems to be more probable. This hypothesis is supported by the work of Kerling (42), who, using Guilliermond's method, stained living cells of potato tubers with neutral red and found that the cell vacuole alone first takes the stain, the remainder of the protoplast being unstained. As soon, however, as the protoplast is damaged, dispersion of the stain becomes general in the cell and a solid, red substance from the stained protoplasm condenses on the surfaces of the starch grains.

If we consider the viruses as extremely small organisms living in the protoplast and transported, as was intimated in the foregoing, by the cytoplasmic connections, it is hardly possible to imagine their sudden growth into x-bodies. That these x-bodies may be centers of condensation products of the protoplast is a more plausible interpretation and is substantiated by the observations of Henderson Smith (33).

Carriers offer a possible answer to this question. When a virus causing symptoms in one host variety and remaining latent in another appears to

be associated with x-bodies in the former and not in the latter, it would seem that these intracellular bodies are a result rather than a cause of the disease.

In her investigations on the viroses of tobacco and potato and on cucumber mosaic in solanaceous hosts, Hoggan showed that the presence or absence of vacuolate bodies, and in some cases the combined presence of vacuolate bodies and striate material have undoubted value for the identification of certain viroses. The tobacco viroses in which striated material occurs were further shown to be closely related by the similar behavior of the viruses when subjected to varying intensities of application of physical agencies, such as heat (Johnson's method).

The "bodies," whose presence characterized a certain virose in one host were nearly, without exception, found to develop in other hosts to which the virus was transferred. Since only vacuolate material or vacuolate bodies and no striate material were found associated with the potato viroses studied by Hoggan, it may be concluded that these viroses are not identical with the three tobacco viroses in which striated material occurs.

It seems, however, that neither shape nor dimensions nor structure of the vacuolate bodies is of diagnostic value. These bodies are all more or less similar in almost all the viroses in which they have been found.

No vacuolate bodies were found in the cells of certain Green Mountain potato plants called "healthy," but that these plants were actually virus carriers was demonstrated when top necrosis developed in plants of Dutch potato varieties bearing grafts from the Green Mountain healthy plants. Cytological investigations in connection with this behavior may be looked forward to with great interest.

CHAPTER 7.

CULTIVATION AND DETERMINATION OF PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE VIRUSES

Symptoms in viroses may be regarded as reactions of the host to the presence of one or more viruses and are therefore influenced by factors that pertain to the host, such as species, variety, age, and environment. This and other reasons have induced workers to determine the characteristics and proprieties of the viruses outside of the plant and to express them in terms of mechanical measurements whenever possible. To this end the methods of cultivation, filtration, dilution, aging, inactivation by heat, x-rays, ultraviolet light, and chemicals have thus far been applied.

Johnson is convinced that the plant viruses have properties of specificity and stability comparable with those of other organisms. The viruses expressed from plants are, however, associated with many other substances, and no one has succeeded in getting rid of these materials by pure culture

of the viruses *in vitro*. It would be to no purpose to mention here the efforts made by different workers to get by filtration, centrifugation, or chemicals, viruses of absolute purity and in a controllable concentration. It is hoped that methods are being developed and will be further developed by which it may be possible in the future to determine physical and other characteristics of viruses *in vitro* independently of uncontrollable variables.

According to Walker (98), a common virus causing the mosaics of cucumber, tomato, and Physalis has not the same properties in juice pressed from infected tomato or ground cherry as in juice pressed from infected cucumber. Extracted from the former two hosts it has, just as the tobacco mosaic, a high resistance to aging, heating, dilution, and treatment with alcohol. Extracted from the latter host, this resistance is considerably decreased.

As has already been pointed out in the foregoing, it would be a misconception to think that there is only one mosaic virus the character of which is changed by each of the hosts in which it lives. It is not surprising that juices of certain plants are toxic to the juice-borne viruses of other plants *in vitro*. Indeed, McKinney (55) proved that the juice of healthy cucumber has a very marked effect in rendering the virus of tobacco mosaic more susceptible to thermal inactivation. That the thermal resistance of cucumber-mosaic virus was increased by a passage through Solanaceous plants needs confirmation by an experiment with real cucumber mosaic. According to Johnson (40), the virus of cucumber mosaic is very different from all the 9 viruses designated by him as tobacco viruses. It differs from them in the symptoms it produces in some standard hosts belonging, respectively, to the Solanaceae, Cucurbitaceae, and Phytolaccaceae. Each of the viruses he worked with produces different symptoms in one or more of these hosts, and these viruses differ also in one or more of the following characteristics: resistance to aging *in vitro*, thermal death point, and resistance to the killing effect of 50 or 60 per cent alcohol and of 0.5 per cent nitric acid.

In some cases it is possible to analyze virus complexes by physical or chemical attack.

Henderson Smith (31) has, from a combination disease in tomato, inactivated the potato-mosaic factor by treatment of the tomato juice with 90 per cent alcohol for one hour and retained the other factor, a virus causing yellow mosaic.

It may be that the question whether Kenneth Smith (82, 84) worked with one or two viruses in his experiments can be solved by attacking the leaves of the tobacco suffering from the needle-induced ring spot by heat, by chemicals, or by drying. According to Valleau and Johnson (96), the American "healthy-potato virus" (probably the top-necrosis virus is intended) does not withstand drying, and by this means they have eliminated this virus from a complex which caused streak in tomatoes.

It should be borne in mind, however, that a classification of all viruses on the basis of their physical and chemical characteristics may never be possible on account of the fact that a great number of them are not transmissible with juice under present methods of experimentation. It is conceivable, of course, that new methods may be devised which would obviate these difficulties.

CHAPTER 8.

A CONTRIBUTION TO THE CLASSIFICATION OF POTATO VIROSES

As a general conclusion from the foregoing it may be stated that present knowledge is insufficient for the classification of plant viruses but that the correlation of different methods offers possibilities. Further, it appeared that the morbid anatomy and physiology of the host is a more or less neglected phase in the study of plant viroses. The study of potato leaf roll, however, shows that by correlation of the histological and physiological methods a better conception of the pathological process can be obtained. The reduced growth of the plant suffering from leaf roll, the stiffness of its leaves, the accumulation of the products of photosynthesis in them, the shortness of its stolons, and the reduction of the yield of tubers could be attributed to disturbed transport; this, in turn, to a diseased condition of the sieve tubes which ends in phloem necrosis. It is the aphid *Myzus persicae* which introduces the virus in the sieve tubes.

At the International Congress of Plant Sciences held at Ithaca in 1926, a Committee for the nomenclature of potato viruses was formed. As a consequence, a standard named collection of American potato-virus material was received by the writer through the courtesy of Dr. Schultz and Dr. Fernow. Previously, a disease resembling what Murphy has called crinkle and what Schultz and Folsom have called rugose mosaic had been isolated by the writer from the old American Cowhorn potato, received from Dr. Cummings. Dr. Murphy, of the Irish Free State, was also kind enough to supply some virus types.

Not only did this rich collection of material give the opportunity of comparing the ever-increasing types of mosaic, crinkle, and streak from the Old and the New World but also a new impetus was given attempts to correlate outward symptoms with phenomena of morbid anatomy.

The viruses from abroad have been transferred through Murphy's method of core grafting on Dutch potato varieties. The present communication will be restricted to the results obtained through this method with some of the potato viroses occurring in America and Europe. Other viroses, *e.g.*, witches'-broom and spindle tuber have not yet been sufficiently investigated.

The discovery that all American varieties, whether supposedly healthy or known to be diseased, are carriers of a virus causing necrosis in certain

Dutch varieties and the comparison of it with another necrotic disease for which certain Dutch potato varieties are carriers led to the differentiation of the virus diseases collectively known as streak into two groups, top necrosis (Lat. acro-necrosis) and acropetal necrosis.

Schultz (76), who must have observed the first of these two diseases after inoculating healthy plants of Dutch potato varieties with inoculum from healthy American varieties, says:

"It appears necessary to assume that the necrosis in question is not the so-called 'streak,' since evidence has shown that the Green Mountain as well as other American varieties used in this investigation is susceptible to this malady."

It appeared justifiable to introduce the name top necrosis for this new disease, and this actually has been done in a Dutch publication (Quanjer and Oortwijn Botjes, 69). And it appears still more justifiable to do this, although for international use acro-necrosis is better suited, since it was found that an internal necrosis radiates from some internal phloem strands into the surrounding tissues, killing the tender tips and extending downward through veins, stems, petioles, and tubers, and killing sometimes the apical eyes of the tubers, also.

The second disease, which came into evidence when healthy-looking plants of some old Dutch varieties were grafted on Green Mountain, is identical with a disease described as streak by Artschwager. According to the latter investigator, it is evident that the lesions referred to as streak are elongated and that "their advance along the stem is acropetal." He continues:

"The lesions are the result of necrotic changes in the cells of the collenchyma and adjacent tissues" and "in advanced lesions the necrotic areas extend through cortex and vascular tissues into the pith."

The writer feels obliged to credit Artschwager (1) with the introduction of a sound pathological concept instead of the often-misused term, streak, and proposes the name acropetal necrosis for the disease characterized by a necrosis beginning in the collenchyma, extending in severe cases centripetally, and advancing acropetally along the stem. There are several related diseases belonging to this type described farther on in this paper.

A further possibility of founding classification and nomenclature on a sound basis is opened by the fact that different potato varieties react differently to the same virus. A virus remaining latent or producing a mosaic in some varieties may cause a well-defined necrotic effect in others. In so far as the writer has followed the matter, he is of the opinion that a certain mosaic of the Zeeland Blue potato produces top necrosis in Paul Krüger and that some mosaics which resemble more closely the rugose type

of Schultz and Folsom produce collenchyma necrosis and belong to the acropetal-necrosis group.

In order to clear the ever-increasing mosaic group, the principle should be followed that a potato virus be named after the histological symptoms it causes in the variety reacting most typically in this respect.

As a consequence of this principle, the names rugose mosaic and crinkle must disappear at least as names of sections, for it seems that all types of rugose mosaic and the greater part of crinkle, when grafted onto certain potato varieties, behave as types of acropetal necrosis. Only one case of mild mosaic (the mosaic in Zeeland Blue mentioned above), after being transmitted to Paul Krüger and other varieties, appeared to produce top necrosis.

Most of what hitherto has been mentioned as mild, intermediate crinkle and interveinal mosaic is here provisionally still classed in the section of anecrotic mosaics; but is may be possible to produce necrosis also with some of these mosaics on certain varieties. That being so, the result of the system now proposed will be to exclude a number of potato mosaics from the group of anecrotic mosaics, just as a *fungus imperfectus* must be classed under the Ascomycetes as soon as an ascigerous form appears to be associated with it.

A disadvantage of this method is that the progeny of a variety suffering from top necrosis or acropetal necrosis has very often such an extended necrosis that differentiation is no longer possible. The disadvantage, however, of basing a classification on the varieties which react with mosaic symptoms will be considered by investigators as still more important, knowing how unsatisfactory it has been in the past to describe an ever-increasing number of slightly differing mosaics.

It is proposed here that the Dutch variety Paul Krüger, called President, in England, be used as a standard variety in other countries as well, since it shows up and differentiates all the viroses with which the present writer has worked. It has rendered excellent service in differentiating acropetal necrosis and top necrosis. A disadvantage is that it behaves as a carrier towards para-crinkle, described by Salaman and Le Pelley (74). For the identification of this disease the variety Arran Victory is needed. Since all the American material appeared to consist of carriers of top necrosis, the comparison of American potato viroses other than top necrosis with European viroses can take place only on varieties that mask this disease, *e.g.*, Green Mountain or Duke of York. Varieties that mask acropetal necrosis are needed for the identification of other viroses which occur in the carriers of this disease. The present state of the classification work at Wageningen is indicated in the following pages, which may be taken as a progress report.

SECTION I: ANECROTIC MOSAIC

No necrosis, no streak, no drop of the lower leaves of other varieties after they have been grafted with the virus-carrying scion; only mottling and more or less wrinkling of leaflets. Some viruses, after being inoculated into different potato varieties, have thus far produced only mild mosaic, intermediate mosaic, crinkle mosaic, or interveinal mosaic. They are both sap- and aphis-transmissible (77, 78) (23), and they have a lower thermal death point than does a virus causing crinkle or rugose mosaic (41). Masking at high temperatures occurs in this group. Vacuolate material and bodies have been found especially in these mosaics; striate material is not present (34).

In case one or more of these viruses should be found as a result of later work to produce a necrotic disease in some host variety it ought, according to this system, to be classed under one of the groups of necrosis.

Aucuba mosaic in potato differs from the viroses mentioned above in that there is no wrinkling at all in the diseased plants and that it is not transmitted by aphids, or only exceptionally so (23, 68).

SECTION II: PHLOEM NECROSIS

Necrosis restricted to the phloem strands, i.e., sieve tubes and companion cells; strong accumulation of carbohydrates in the leaves. This necrosis is only visible with the high-power objectives (enlargement 100 or more). In the plants infected in the current season, the necrosis is faint and can be detected in some sieve strands only; in the progeny of such plants "perpetuation" symptoms appear and the necrosis is distinct in a much greater number, generally more than half, of the intraxylary and extraxylary phloem strands (compare the necrotic phloem strands in figure 1, B, with the healthy phloem strands in figure 1, A).

The virus causing phloem necrosis thus far appears not to be sap-transmissible. It can be transmitted by grafting; and, in nature, is transmitted by *Myzus persicae* (61) (77, 78) (23) (58), and (83).

The accumulation of carbohydrates is a consequence of the pathological condition of the sieve tubes, and this condition ends in phloem necrosis (66) and (93). No x-bodies have been found (34).

In European potato varieties, the necrosis appears in the phloem of the foliage and stems, only; but in the American variety Green mountain it also occurs in the phloem of the tubers (Figs. 1, C, and 8, A) of the current season where it is visible to the naked eye (net necrosis of Gilbert, 26). That the American leaf-roll virus is identical with the European was proved by Elze and Quanjer (24).

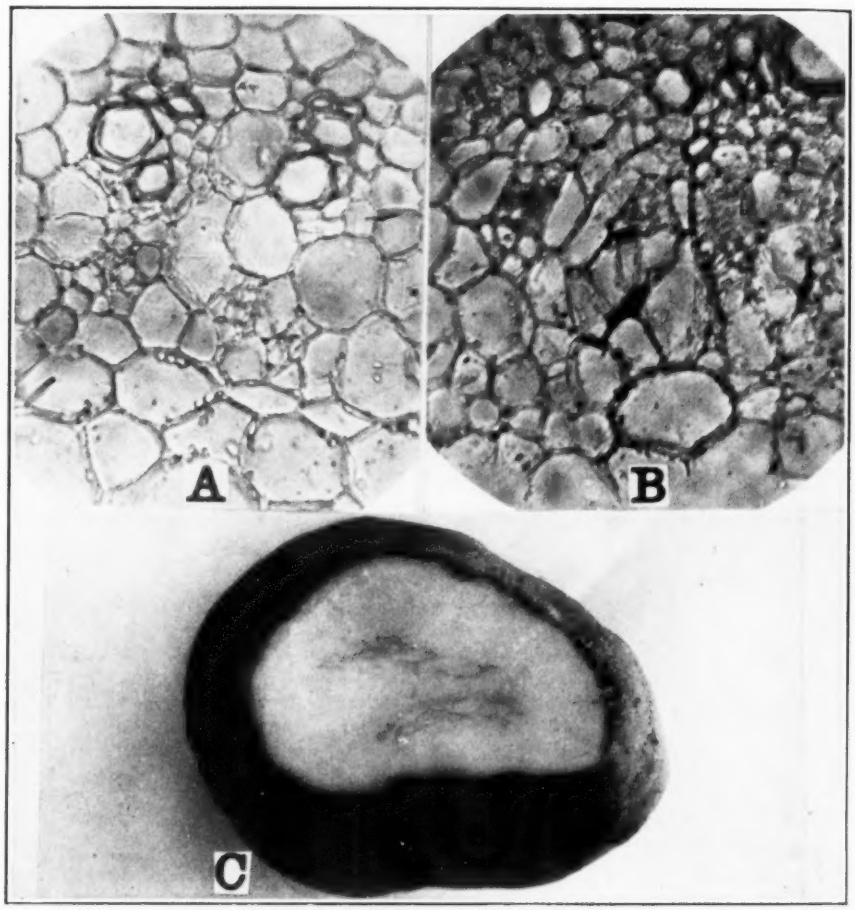

FIG. 1. A. Normal phloem as it occurs in healthy potato plants or plants attacked by anecrotic mosaics. Two internal phloem strands are seen in cross section, surrounded by pith cells and, at the periphery, by some xylem vessels. B. In the midst a necrotic phloem strand typical of potato plants suffering from leaf roll. The necrosis is restricted to the sieve tubes and companion cells. It occurs in the internal as well as in the external phloem. C. Tuber of American potato variety (Green Mountain) taken from a plant, infected by leaf roll from a European variety (net necrosis of Gilbert, 26).

SECTION III: TOP NECROSIS (ACRONECROSIS, LAT.)

Necrosis radiating from only a rather small percentage of the internal phloem strands, almost never from the external phloem strands, into the surrounding parenchyma, this in turn surrounded by a cork cambium, except in the tender tips, which are soon killed; occurring in foliage, stem, and tubers. In the tender leafy tips an internal necrosis appears and is

at first localized in certain phloem strands inside the xylem, from which centers it soon progresses radially in the surrounding parenchyma and appears at the surface. The young leaves of the tips then develop necrotic spots (Fig. 4, A). These necrotic spots of leaves, petioles, and apical stem end later coalesce to the extent that the tender tips of the shoots are entirely killed (Figs. 5, A, and 6, A).

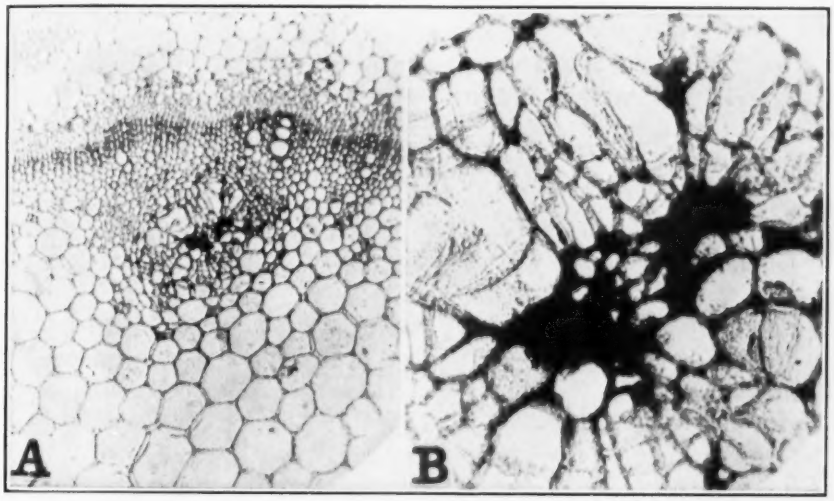

FIG. 2. A. Necrosis radiating from the internal phloem in the surrounding parenchyma as it occurs in some Dutch, German, and English potato varieties that have been grafted with scions of apparently healthy plants of the American varieties Green Mountain, Irish Cobbler, and Rose 4, the Scotch variety Duke of York, and the French variety Jaune d'Or. This necrosis is the histological characteristic of the disease called top necrosis or acronecrosis in this paper. B. Portion of A enlarged. By the formation of cross walls a cork cambium of flat cells is being formed that separates the necrotic cells from those still sound.

Immediately below the dead tips the strands of necrotic tissue consisting of phloem and adjacent parenchyma cells are surrounded by a layer of cork cells. In figure 2 it can be seen that by the formation of cross walls a cork cambium of flat cells has arisen. In the leaves directly below the affected tips, necrotic spots, only, are found; the leaves still lower down remain free from spots. Although the phloem necrosis found in plants suffering from leaf roll is restricted to the sieve tubes and companion cells and can be seen only with high-power objectives, the phenomenon described here can be seen with a pocket lens and, in the tubers, it is plainly visible to the naked eye, appearing as large blotches. In the tubers, also, it starts from the internal phloem and sometimes the apical eye is killed (Fig. 7, A).

The top-necrosis virus is sap-transmissible; but, whether it is transmissible also by *Myzus persicae* is doubtful. Thus far nothing has been seen

which points to the possibility of transmission by insects (*cf.* Elze's paper, in this number of PHYTOPATHOLOGY).

No x-bodies have been found by Hoggan in American healthy potatoes carrying this virus.

After grafting scions of apparently healthy Green Mountain, Irish Cobbler, Rose 4 (American), Duke of York (Scotch), and Jaune d'Or (French) onto several Dutch and German varieties, top necrosis showed up in the grafted plants. The apparently healthy scions used in this experiment were carriers of a virus that produced in both the Dutch and the German varieties a disease that had never before been observed in Holland under field conditions. (It has, however, been observed by the writer in the "massif central" of France.)

Top necrosis may be caused by more than one virus (69). For the purposes of this discussion, the group as now known may be divided tentatively into two types, A and B. This division is somewhat suggestive of the classification of the rusts into biotypes.

Type A includes: (1) A virus that is latent in the Scotch variety Duke of York and is semilatent in Zeeland Blue, causing a mosaic in this variety; (2) a virus that may be identical with (1) but is known to be latent in the American varieties Green Mountain, Irish Cobbler, and Rose 4; and (3) a virus recently found to be carried by the French variety Jaune d'Or and which may possibly be identical with (1) and (2) of this type.

Type B is represented by a top-necrosis virus that is carried by some strains of the Dutch varieties Monoeraat and Roode Star and is distinct from the viruses of type A, since it produces necrotic symptoms in Duke of York, a carrier for the viruses of type A, and in Zeeland Blue, a variety in which the viruses of type A are semilatent and cause a mosaic.

The virus of yellow dwarf may perhaps be included in this section. According to the description of Barrus and Chupp (5), this disease resembles top necrosis. From a histological point of view their description, however, is very incomplete. It differs from the viruses of types A and B in that the symptoms it produces appear in inoculated plants of the variety Green Mountain when grown at high summer temperatures, but fail to develop in this variety when the inoculated plants are grown in the cool summer climate of Holland. Fernow, in his letter accompanying the tubers sent from America, wrote that the development of this disease is favored by growing the plants at 15° C., until they are 5 to 6 in. high and then transferring them to a temperature of 25° C.

That Hoggan did not find x-bodies in healthy American Green Mountain does not seem to exclude the possibility of their occurrence in varieties showing symptoms of top necrosis (*cf.* 6).

SECTION IV: ACROPETAL NECROSIS

Necrosis chiefly in the collenchyma of the leaf veins, petioles, and stems, in cases extending gradually to other tissues, no restriction by a cork cambium. The advance along the stem is acropetal. Dropping of lower leaves. Rugose mosaic (Schultz and Folsom) and a part of the diseases collectively known as crinkle and streak are characterized by this type of necrosis.

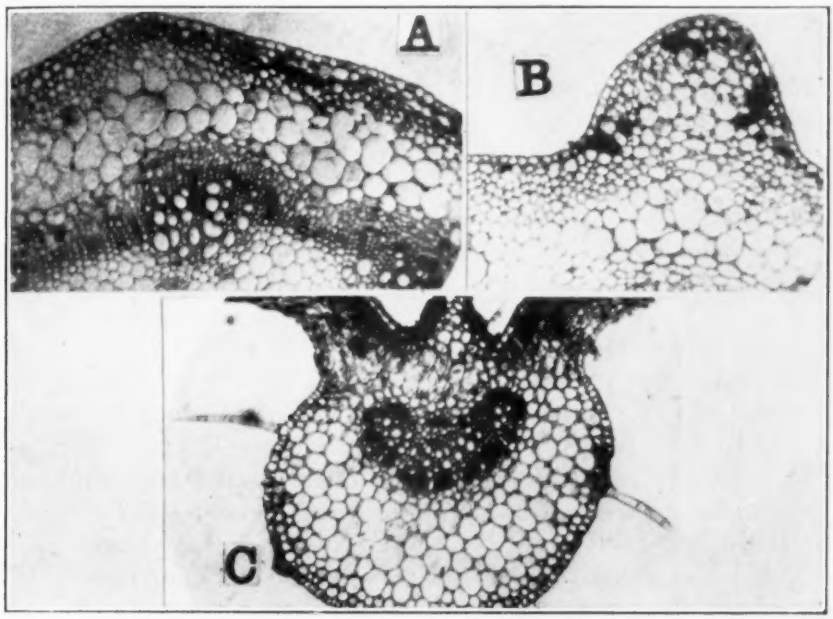

FIG. 3. A. Necrosis beginning in the collenchyma of the stalks as it occurs in some Dutch and American potato varieties that have been grafted with scions of apparently healthy plants of the old Dutch varieties Zeeland Blue, Botergele, Bloemgraafjes, and Gladblaadjes, and of the new Dutch variety Thorbecke. This necrosis is the histological characteristic of the disease described as acropetal necrosis in this paper. Some related but not identical forms of this disease occur in Europe and America. They have been described as streak, stipple streak, crinkle, and rugose mosaic. B. The same "collenchyma necrosis" in the "wing" of the stalk. C. The same necrosis as that found in the collenchyma adjacent to the veins of the most strongly downward-curved parts of the leaves of plants suffering from crinkle or rugose mosaic. The strongest necrosis is visible in the collenchyma adjacent to the upper side of the vein; the necrosis also is visible in two spots of the collenchyma adjacent to the under side of the vein.

In most of the types described as crinkle and in rugose mosaic, the same necrosis of the collenchyma is found as is typical for acropetal necrosis, but it is largely restricted to the collenchyma adjacent to veins of those parts of the leaves that are most strongly curved downward (Fig. 3, C);

the necrosis does not extend to the petioles and stems, nor do the tubers of the plants suffering from rugose mosaic or crinkle show distinct effects of the disease. The perpetuation symptoms in crinkle may be somewhat more severe than the inoculation symptoms, but they are of the same type and are annually recurrent. Yet, rugose mosaic, crinkle, and streak are very closely related; in both, the lower leaves, in which the collenchyma necrosis is most developed, drop prematurely, and the same virus in one variety may produce a rugose mosaic or crinkle and in another variety a streak.

The virus causing rugose mosaic has a rather high thermal death point (41).

No masking of symptoms by high temperatures occurs in this group. No x-bodies have been found (34).

Quanjer and Oortwijn Botjes (69) have described different types of this group occurring in Holland. In type A, Atanasoff's acropetal necrosis, necrotic spots adjacent to the veins and consequently angular appear in the lower and middle leaves, not on the top leaves (Figs. 4, B; 5, B; and 6, B), the necrosis beginning in the collenchyma of the leaf veins, leaf stalks, and stems (Fig. 3, A and B); superficial necrotic areas appear also on the tubers (Fig. 7, B).

The second-year plants as a rule are small, show severe crinkling, and are brittle as glass; their leaves are spotted, their veins are brown, their lower leaves drop off early in the growing period, and their stems are heavily striped. The necrosis extends to the interior and the plants die prematurely; the tubers are small and the necrosis extends deeper in them than in the first-year tubers. They die, as a rule, during storage.

This disease shows complete symptoms in the varieties Paul Krüger, Duke of York, and Green Mountain; it occurs in a semilatent form resembling Murphy's crinkle or Schultz and Folsom's rugose mosaic in the varieties Bravo and Rose 4 and in a still more suppressed form as mild rugose mosaic in the variety Eigenheimer. It is present in an entirely masked form in the old Dutch varieties Zeeland Blue, Botergele, Bloemgraafjes, and Gladblaadjes. A somewhat more virulent type of this disease occurs in a wholly masked form in the Dutch variety Thorbecke.

Attenuation of the streak virus carried by Zeeland Blue has been brought about by the writer by allowing it to pass through a potato seedling which itself acted as carrier. For this reason differences in severity of the symptoms are not to be regarded as a reason for classifying a virus as a different type.

A rugose mosaic in Cowhorn from America behaves in Paul Krüger as an acropetal necrosis, which is not distinguishable from that of type A.

What seems to be another type (B) of acropetal necrosis has been found by Oortwijn Botjes in the fields in the variety Noordeling. In plants of

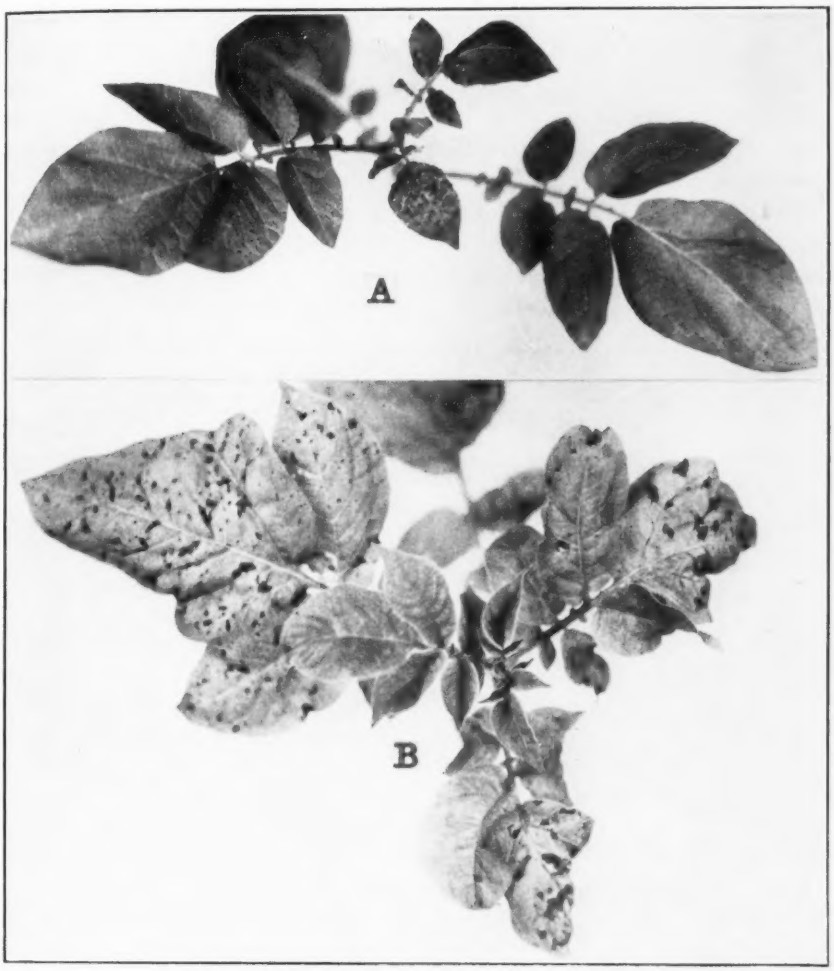

FIG. 4. A. Top necrosis or acronecrosis showing up in the tender tips of the lateral shoots of Paul Krüger (President) the stalks of which have been grafted with apparently healthy plants of Green Mountain. The necrotic spots developed within about 12 days after the grafting took place. This disease is characterized by necrosis radiating from the internal phloem in the surrounding parenchyma (Fig. 2 A. and B.). B. Acropetal necrosis showing up in the expanded leaves of Paul Krüger, which have been grafted with apparently healthy plants of Zeeland Blue. The necrotic spots developed about 12 days after the grafting took place. They extend from the collenchyma adjacent to leaf veins, petioles, and stems.

Bravo, bearing grafts of the disease occurring in the field, a type of crinkle appears somewhat different from that produced by grafting Bravo with scions of Zeeland Blue. The rugosity in the latter type of crinkle is more

severe than in the former; also, there is more mottling in the latter than in the former. When scions of Zeeland Blue are grafted on Noordeling the resulting symptoms include more leaf drop than is typical of the diseased Noordeling in the field. Each of these two viroses, which have many points in common, retains its individuality unchanged through a long term of years.

Also considered to belong to this group is rugose mosaic in Green Mountain. A difficulty in diagnosing it on the differential host Paul Krüger arises through the appearance of top necrosis in the latter. The transmission on Duke of York, which itself is a carrier of top necrosis, resulted in

FIG. 5. A. Top necrosis or acronecrosis as seen in the differential host variety Paul Krüger 18 days after it has been grafted with the carrier Green Mountain. The tops of the lateral shoots, developing beneath the graft, are killed. B. Acropetal necrosis as seen in the differential host variety Paul Krüger 18 days after it has been grafted onto the carrier Zeeland Blue. The expanded leaves of the side shoots developing under the graft are covered with necrotic spots beginning at the collenchyma of the leaf veins, petioles, and stems. These leaves are killed and remain hanging down from their point of attachment.

the appearance of rugose mosaic in this variety. Also a crinkle in the variety Up to Date, from Ireland, behaved as rugose mosaic in Paul Krüger.

This section, no doubt, will be found to contain still further types and a classification in A, B, etc., must be looked upon as provisional in character. How far Salaman's crinkle A, para-crinkle, and streak A are related to the types thus far studied must be determined by comparison on standard varieties.

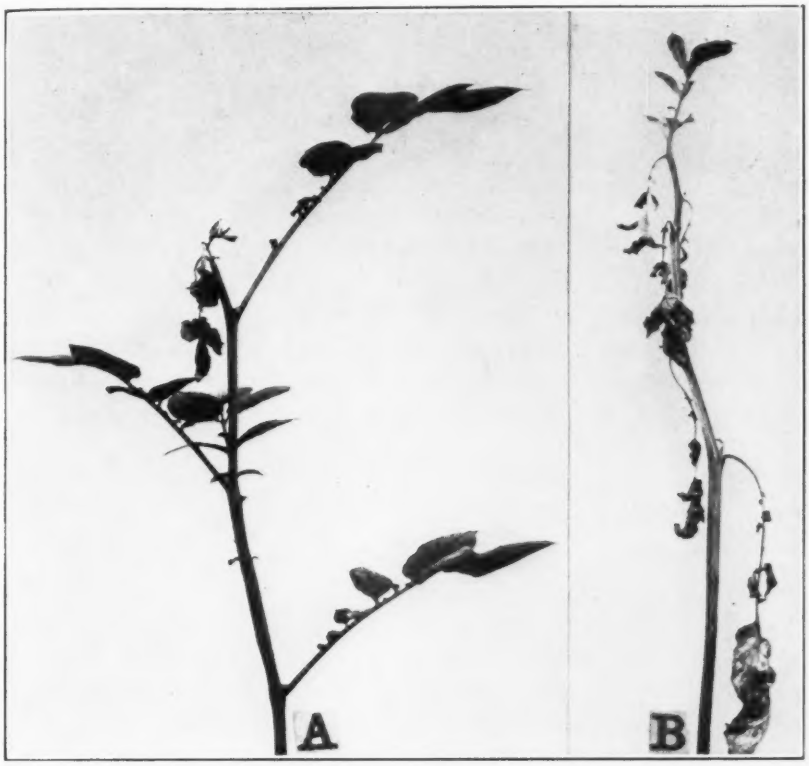

FIG. 6. A. A lateral shoot of a Paul Krüger plant 24 days after the main stalk has been grafted with Green Mountain. The top necrosis has killed the tender tip as well as a nearly developed leaf. It extended to the lower leaflets of a fully expanded leaf; necrotic spots are developing on the other leaflets of this leaf. The lower leaves remain free from spotting. B. A side shoot of a Paul Krüger plant 24 days after the main stalk has been grafted with Zeeland Blue. The leaves of this side shoot were covered with angular necrotic spots beginning at the collenchyma of the ribs and extending over the petioles and the stem. The youngest leaves at the top remain free from symptoms, as is typical for acropetal necrosis.

Whereas the types A and B of top necrosis described above differ from each other in the range of varieties that show symptoms and are, in this respect, comparable to the biotypes of the rust fungi, the types of acropetal necrosis mentioned here show, besides differences in the range of reacting hosts, also slight differences in the type of symptoms produced and for this reason are comparable to the biotypes of *Colletotrichum Lindemuthianum*.

It would be extremely interesting to know if the relation of the members of the acropetal-necrosis section also can be proved by Johnson's methods.

SECTION V: PHLOEM PARENCHYMA NECROSIS OF THE TUBER (PSEUDO-NETNECROSIS)

Necrotic spots in the storage parenchyma next to the external and internal phloem of the tubers only; no foliage symptoms. The disease is perpetuated by the seed tubers. The necrotic areas are spot-like rather than blotchy (as in top necrosis). The disease does not resemble netnecrosis (*cf.* Fig. 8, A and B) with which it has been confused by Atanasoff (4), hence the name pseudo-netnecrosis, which the writer originally gave to the disease, but which has the disadvantage of being derived partly from English and partly from Greek. Therefore, the name phloem-parenchyma necrosis of the tuber may have advantages for international use. Phloem-parenchyma necrosis of the tuber is sap- and aphis-transmissible (68). High temperature during tuber storage encourages the development of the symptoms.

A disease called "vererbliche Eisenfleckigkeit" by Fruwirth (25) is probably caused by the same virus.

SECTION VI: CONCENTRIC NECROSIS OF THE TUBER

Necrotic spots in the storage parenchyma demonstrating themselves on the cut surface as concentric brown rings arising from some point on the skin, often a lenticel. This disease is not perpetuated by the seed tubers. The rings (Fig. 8, c) are composed of necrotic cell groups which resemble in their microscopical features those inside and outside the xylem in phloem-parenchyma necrosis (42). The cell groups, however, are arranged in concentric rings in concentric necrosis. Infection takes place from the soil, evidently through some of the lenticels (*cf.* Chapter 1). There is no transmission by seed tubers, (92), (4), (67).

The disease is already developed at the end of the growing period, hence the wound-cork tissue around the necrotic spots is more extended than in phloem-parenchyma necrosis.

The disease has the popular name "kringerigheid" in Holland. It is known in Germany under the name "Pfropfenbildung" and a part of the German "Eisenfleckkeit" and the American and English "internal brown spot" or "internal rust spot" may be identical with it. In the French paper of Schwellengrebel the disease is called "Maladie des tâches en couronne."

DISCUSSION

One can hardly deny the existence of a whole world of organisms extremely small, invisible or hardly visible, and not yet cultivable apart from the living host, that cause plant viroses. The multiplication of all the viruses in their hosts, the occurrence of what seem to be species, varieties,

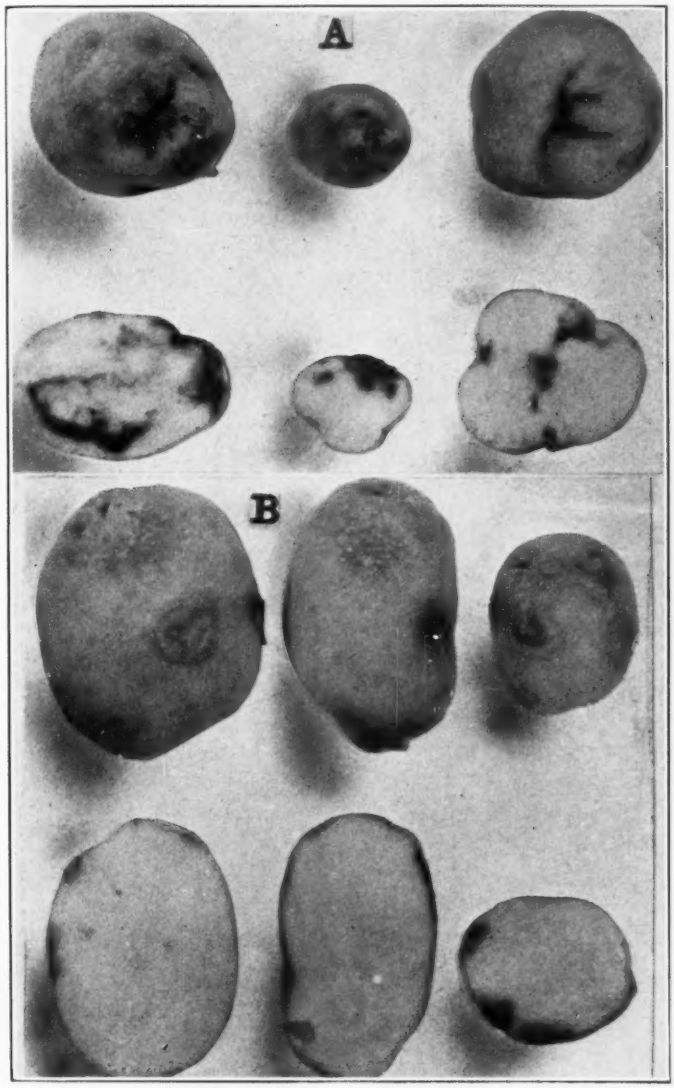

FIG. 7. A. Above, the tubers of originally virus-free plants of the varieties Paul Krüger, Bevelander, and Kerr's Pink, dug 3 to 4 months after the mother tubers had been core-grafted with an apparently healthy Green Mountain carrying top necrosis or acronecrosis. Below, the same tubers, cut. The necrosis radiates from the internal phloem and may kill the apical eye. B. Above, the tubes of originally virus-free plants of the varieties Paul Krüger, Duke of York, and Green Mountain, dug 3 to 4 months after the mother tubers had been core-grafted with apparently healthy Zeeland Blue carrying acropetal necrosis. Below, the same tubers cut. The necrosis is superficial, agreeing with what can be seen in the stalks (where it begins in the collenchyma).

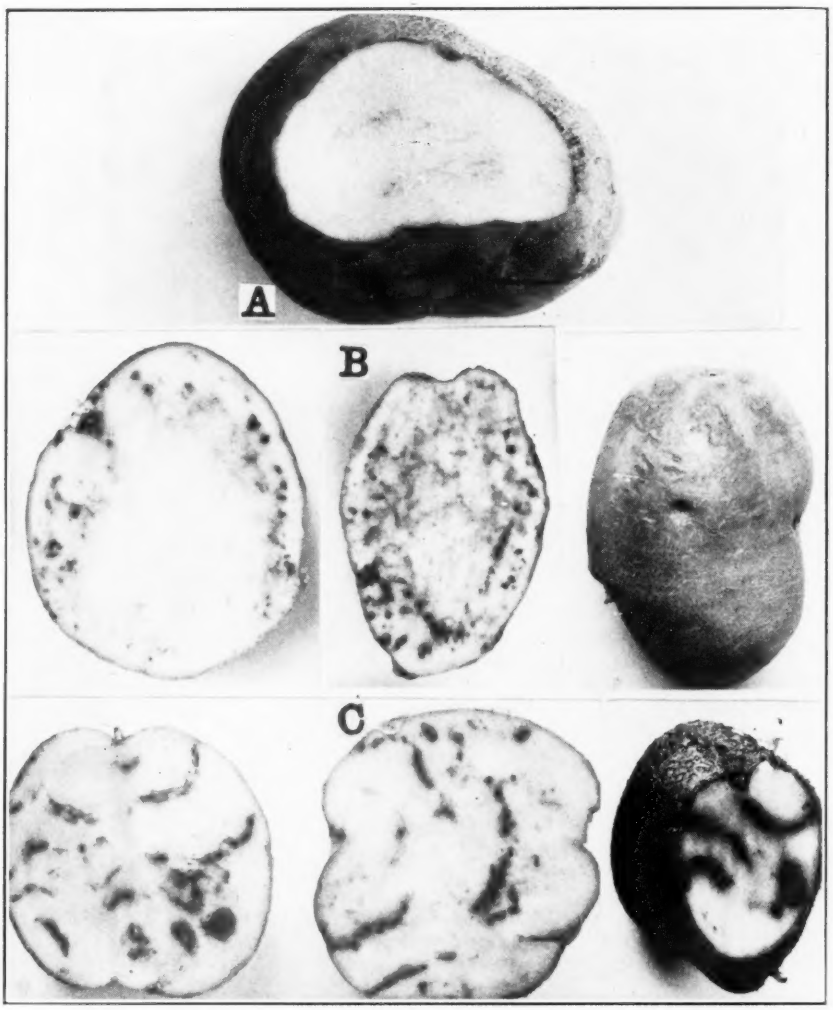

FIG. 8. A. Netnecrosis (phloem necrosis in the tubers of Green Mountain) for comparison with B and C. B. Pseudo netnecrosis, or phloem parenchyma necrosis of the tuber, at the left and in the middle in variety Roode Star, the necrotic spots are in the phloem parenchyma inside and outside the xylem ring. At the right in variety Paul Krüger, where the necrotic spots are visible at the surface near the heel end. C. Concentric necrosis of the tuber at the left in the Dutch varieties Alpha and Roodeschoolsche Jam, at the right in the German variety Direktor Johansen, where it is visible at the surface.

and biotypes, the phenomena of attenuation, and increase of virulence suggest their parasitic nature. Also, the "waiting period" of some of the viruses in the bodies of the transmitting insects, evidence for which has been

furnished by Severin, Kunkel, Elze, Storey, and Smith, is, in some cases at least, suggestive of parasitism. This holds true especially for the virus of aster yellows with its long incubation in *Cicadula sexnotata*. The impossibility of growing the viruses *in vitro* carries with it the impossibility of differentiating between them by means of chemically different nutrient media or by differing chemical reactions on identical media, as is done with bacteria. This is the reason why identification and differentiation are dependent largely on symptomatology.

Earlier descriptions of the morbid symptoms in plants suffering from viroses are frequently not specific, detailed, or accurate enough. For this and other reasons, Johnson presented a classification of some tobacco viroses chiefly on the basis of physical properties determined in the virus-containing juices and their behavior towards treatment by chemicals. Later he applied the same method of classification to some of the potato viroses. While such direct methods offer great possibilities, especially for the knowledge of the nature of the viruses concerned, it can not be expected from these methods that they give any information on the properties of the viruses which interest us most, *i.e.*, their properties as pathogenes.

It has here been shown that the indirect method of symptomatology can be improved by histological and physiological refinement in connection with appropriate hosts, appropriate transmission, and control of environmental conditions and that a classification and nomenclature of some potato viruses, at least, is possible on the basis of well-defined pathological conceptions. To terminate the indiscriminate use of confusing names for an ever-increasing number of mosaics, crinkles, and streaks, it is proposed to adopt the nomenclature presented in this paper and to develop it further.

Furthermore, it is suggestive for the study of the nature of the viruses concerned to observe that, at least in those varieties which are liable to necrosis, they select different plant tissues on which to exert their enzymes and toxins. The leaf-roll virus acts on the sieve tubes and companion cells; the top-necrosis viruses, on the parenchyma surrounding the internal phloem; the viruses causing acropetal necrosis, on the collenchyma first; the virus causing phloem-parenchyma necrosis, on the storage parenchyma of the tubers, only; while some of the mosaic viruses thus far seem only to injure the mesophyll of the leaf without causing actual necrosis.

There is an interesting morphophysiological problem in the correlation of a necrosis radiating from the internal phloem with the killing of the tender tips and in the correlation of a necrosis extending from the collenchyma with a killing effect advancing acropetally. The solution of this problem must be the aim of future work.

The difference in plant tissues attacked must be due to chemical differences in the causal agencies. Although classification of plants and

animals, as a rule, is based on morphology, it is in some cases not objectionable to classify them on a chemical basis as well. Johnson has done this, for his diagnostic features of the property type must be attributed to chemical characteristics of the viruses concerned.

And in a more general way we may take it as granted that even form in the vegetable and animal kingdom is a function of chemical processes. Thus, chemical agreements or differences may be of taxonomic value. This being true, the basing of a classification and a nomenclature of viruses on clearly definable necrotic effects, typical for each of them, may be considered a valid principle in virological studies.

SUMMARY AND CONCLUSION

From the discussion in the foregoing on the principal methods of identification, differentiation, and classification of plant viruses, and their respective advantages and disadvantages, we may conclude that our present knowledge is insufficient for classification of plant viruses and that the method of morbid anatomy and physiology thus far is somewhat neglected.

A standard named collection of potato-virus material from the leading workers of the Old and the New World being available, it was attempted to find a new basis for the classification and nomenclature of the viroses of the potato—based on such characteristics as can be recognized by everybody able to use a microscope.

The principle has been followed that a potato virus should be identified, named, and classified after the morbid effect it has on a variety that shows clearly definable internal symptoms. None of the Solanaceae other than the potato have been used to test the viruses for this purpose. For international corroboration and agreement on nomenclature, a stock of the same differential host varieties should be grown in a virus-free state at all research stations where potato-virus studies are in progress. One of the best differential hosts is the variety Paul Krüger, called President, in England.

To avoid further trouble resulting from the use of descriptive names for an ever-increasing number of mosaics, crinkles, streaks, and spottings, the writer proposes the following as an international nomenclature for the potato diseases of the virus type:

Anecrotic mosaics (the result of later work may be that some of the members of this section must be classed under one or more of the groups of necrosis).

Phloem necrosis (containing thus far one disease, called leaf roll, or as inoculation symptom in tubers of Green Mountain, net necrosis).

Acronecrosis (or top necrosis, a disease produced by healthy-potato virus occurring in most of the American standard varieties and in a Scotch

and a French variety. The disease shows up after grafting these carriers on to a differential host).

Acropetal necrosis (containing rugose mosaic and a part of the diseases hitherto called crinkle and streak. Viruses belonging to this group may be latent in some European varieties, which consequently behave as carriers).

Phloem-parenchyma necrosis of the tuber (*Pseudo-netnecrosis*) (no foliage symptoms belong to this disease, which is perpetuated by the seed tubers).

Concentric necrosis of the tuber (no foliage symptoms. This disease is not perpetuated by the seed tubers; infection takes place from the soil, obviously through the lenticels).

Short descriptions of the histological features characteristic for each of the six respective sections are found in the eighth chapter.

It is hoped that the grouping of potato viroses after the system outlined in this paper will, in future investigations, prove consistent with results obtained through other ways of approaching the problem, and that the system, still in a tentative stage, may appear applicable to other viroses not yet sufficiently studied histologically.

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THE INHERITANCE OF THE REACTION OF MAIZE TO GIBBERELLA SAUBINETII[1]

K. G. McINDOE

INTRODUCTION

The use of resistant varieties in the control of root-rotting diseases of maize is universally recognized as the method giving most promise. A prerequisite to the production of such varieties is the isolation of resistant selfed lines, the resistance of which is then to be combined with advantageous characters of other lines in producing the desired varieties. It is in the combination of such lines that knowledge of the manner of inheritance of reaction to the disease becomes useful. The present investigation was conducted mainly with the intention of obtaining this information.

Gibberella saubinetii (Mont.) Sacc. of late years has been recognized as largely contributory in causing root and stalk rots of maize in the Corn Belt of the United States. Investigation of the disease in Minnesota has mostly centered around this pathogene. Its deleterious effect is most obvious upon the seedling, and, according to the extent of injury, the reddish-brown lesions may be purely local on coleoptile, coleorhiza, mesocotyl, and primary and seminal rootlets, or may involve the greater part of the developing embryo. In extreme cases, the mesocotyl (the structure between the base of the coleoptile and the attachment of the scutellum) may be entirely rotted through, in which case the food supply to the young leaves is cut off and the plant wilts. Early production of adventitious roots from the first node may enable the plant to avoid destruction, even though the mesocotyl may be well rotted. Such plants may appear to be perfectly healthy above the ground and, indeed, may remain so. In others the adventitious roots do not appear early, the leaves do not receive adequate nourishment, and the plants either die or linger on in an attenuated fashion. The study of the inheritance under discussion necessitates the isolation of selfed lines of maize which exhibit decided differences in their degree of resistance. At the initiation of the investigation herein described such differences had

[1] This study is a phase of the investigations conducted by the Division of Agronomy and Plant Genetics, in cooperation with the Division of Plant Pathology and Botany, on the general problem of disease resistance in plants. A thesis presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The author wishes to express his indebtedness to Dr. H. K. Hayes for direction and criticism throughout the study. To Dr. E. C. Stakman and to Dr. J. G. Leach his thanks are also due for criticism and suggestions regarding the manuscript. Published with the approval of the Director as Paper No. 973 of the Journal Series, Minnesota Agricultural Experiment Station.

already been broadly established by greenhouse experiments conducted by the Divisions of Agronomy and Plant Genetics and Plant Pathology and Botany, at the Minnesota State Agricultural Experiment Station. The lines thus distinguished were therefore available. In dealing with such a quantitative character as disease reaction, the experimenter is indeed fortunate if he has immune parental stock, *i.e.*, lines homozygous for complete resistance. The material available in this study was in no way remarkable in possessing this desirable attribute; in fact, as will be shown later, certain environmental conditions would transform apparent high resistance into complete susceptibility. However, under the circumstances, resort was had to the best that offered, remembering that the variability of the reaction was likely to preclude any simple explanation of the results.

PREVIOUS INVESTIGATIONS

The more strictly pathological phases of cereal root-rotting diseases are naturally fundamental to any study of the inheritance of such diseases. Symptomatology of the diseases, identification of the pathogenes involved, their relative virulence, and their distribution are aspects of the matter that seem scarcely relevant in this paper, and consequently detailed reference to such work may well be omitted. Suffice it to say that the recognition of the predominant influence of *Gibberella saubinetii* by Hoffer, Johnson, and Atanasoff (5), as well as by Dickson, Johann, and Wineland (3), in causing root rot of maize focussed attention upon its potentialities.

The study of seedling reaction of maize and wheat to *Gibberella saubinetii* hinges largely about the work of Dickson and Holbert. Dickson (1, 2) demonstrated the relation between the environment and the expression of disease symptoms. He showed that the temperature of the soil is the most important single factor determining the extent of seedling blight and that, while temperatures of 12° to 28° C. favored the blighting of wheat, maize seedlings were more subject to attack between 8° and 20° C. Resistant and susceptible strains were isolated, and, while the latter succumbed at temperatures lower than 24° C., the former were not attacked until grown at temperatures lower than 16° C. Dickson's explanation of the difference in reaction of wheat and maize involved consideration of the changes in metabolism of the host produced by differences in temperature, so that the manifestation of disease was conditioned more by host reaction than by fungus response to the varying temperatures. At temperatures unfavorable to the growth of the host, its metabolism became "unbalanced" and predisposition to attack was increased.

The same author (1) also found that the extent of blighting of wheat seedlings—other factors being favorable for infection—was directly propor-

tional to the amount of infestation, *i.e.*, to the amount of inoculum in contact with the seedling. With wheat, also, he showed that low soil moistures increased the percentage of blighted seedlings at all temperatures.

That factors other than intrinsic metabolic processes may be operative in conferring apparent resistance or susceptibility has recently been suggested by Peterson (9). While admitting the influence of such intrinsic processes, he would prefer to attribute the apparent resistance of seedlings to the power possessed by them of producing a functioning nodal root system before the mesocotyl becomes completely rotted by the fungus. The leaves of such strains as are not capable of the early production of nodal roots are wilted quickly as a result of the severance of their food supply.

It is probable that a combination of both intrinsic resistance and such "avoidance" of destruction is active in producing the final result. It becomes apparent, however, that the influences which determine the estimated degree of resistance are by no means simple and that we are dealing with a complex reaction.

A further factor that may be connected with resistance of the mature plant has been suggested by Hoffer and Carr (6). Their contention is that plants which contain the largest quantities of aluminum and iron salts appear to develop the most severe cases of root rot, provided other conditions are favorable for infection. Deficiency of available phosphates also seems to favor fungous infection. They suggest that the power of selective absorption of these metals may be hereditary and thus influence the incidence of disease.

Dickson and Holbert (4), in preliminary studies on the inheritance of disease reaction, come to the only conclusion appearing in the literature on this subject. They state that "first-generation hybrids between (these) resistant and susceptible strains are susceptible to seedling blight at all temperatures. That is, susceptibility is dominant in such crosses." They give no experimental details in support of this statement.

EXPERIMENTAL MATERIALS

The parental cultures employed in the inheritance investigations consisted of 27 selfed lines of maize, comprising 3 varieties, viz, 9 lines of Minnesota 13, 10 lines of Rustler, and 8 of Northwestern Dent. All these lines had been selfed previously from 7 to 14 years and therefore could be assumed to be reasonably homozygous. Within the lines of each variety, all possible crosses had been made in 1927, 1928, and 1929, so that, with the exception of a very few crosses unavoidably lost, almost all these F_1 crosses were available for testing in the greenhouse. Wherever possible, the most recently produced seed was used, but, if not available, recourse was had to

seed from the previous year. A total of 105 F_1 crosses were thus employed. Preliminary tests were conducted in the winter of 1928–29 on parental lines and F_1 crosses, but, on account of variable greenhouse temperatures, the results were not considered sufficiently reliable. However, these experiments adequately indicated broad differences between the various lines, and, with this knowledge, certain crosses were selected for further study in the F_3 generation.

In the winter of 1929–30, therefore, there were available the parental lines, the F_1 crosses, and certain F_3 lines obtained by growing the F_2 in the field in 1929 and self-pollinating plants at random. The crosses from which these F_3 lines originated were selected on the basis of the reaction of the parents giving rise to them. Five such crosses were selected as follows:

Minn. 13	43 × 46	Resistant × Susceptible
Minn. 13	43 × 47	Resistant × Susceptible
Minn. 13	49 × 50	Susceptible × Intermediate
Rustler	58 × 60	Susceptible × Susceptible
N. W. Dent	64 × 66	Intermediate × Susceptible

It was somewhat unfortunate that of all the lines tested only one, 43, could be called resistant. Consequently a cross Resistant × Resistant could not be selected.

Throughout the study, a single physiologic form of *Gibberella saubinetii* was used, which originally was obtained by the Department of Plant Pathology from L. R. Jones of Wisconsin and designated by Tu (10) as *Fusarium graminearum* form I. Of the three forms identified by Tu, this form was found by him to be most virulent in causing head blight of wheat, and preliminary experiments conducted in connection with the present investigation indicated that its pathogenic properties were similarly manifested in producing root rot of maize. It was also distinguished by its ability to form perithecia in pure culture, a property not displayed by the other two forms.

EXPERIMENTAL METHODS

As has already been indicated in the introductory section, environmental conditions modify considerably the expression of disease symptoms. Consequently, reduction of variability in such conditions becomes an essential if reliable bases of comparison are to be made. Temperature, as well as soil moisture, light intensity, and amount of inoculum are the chief factors to be considered, and, of these, variation in temperature creates the greatest disturbance.

Absolute control of temperature in a large greenhouse is of course quite impossible; nevertheless, by the use of radiators equipped with blowers

automatically regulated by thermostats, it was found that the air temperature could be controlled during the winter months within the limits of about 3° C.

By maintaining the air temperature as near as possible to 20° C., the soil temperature as a rule approximated 15° C. This temperature was sought, for, though low enough to permit manifestation of infection in the great majority of lines, it still allowed sufficient growth of the plants. With the variation in air temperature, the soil temperature naturally fluctuated concomitantly, though never to the same extent, owing to lag. As a general rule, the variation in soil temperature was rarely more than 2° C. about the selected temperature (15° C.). A diurnal fluctuation was always present, with the highest temperatures from noon to 4 p. m. and the lowest from 4 a. m. to 8 a. m. This fluctuation was particularly accentuated if the sun happened to shine during the day. The air and soil temperatures were recorded on a thermograph actuated by two thermocouples, one buried 2 inches in the sand and the other supported about 4 inches above the surface. Owing to the fact that the radiator in each greenhouse was situated at one end of the house, it was necessary to maintain air circulation by judicious placing of electric fans about the floor. Even with this precaution, it was frequently found that an absolutely uniform distribution of temperature was difficult of attainment, so that local regions of the sand benches tended to show divergences of temperature of about 2°. This divergence may seem small, but its effect as expressed by the survival of the plants was plainly noticeable.

The seedlings were grown in clean, freshly dug sand of a fairly coarse texture, which could be considered reasonably free from pathogenic organisms likely to vitiate results. This sand was watered every day and thus kept quite moist. Two greenhouses were used, and each contained two benches running longitudinally at waist height, and about 6 inches in depth. One bench in each house was used habitually for inoculated seeds and the other planted with seeds of similar strains treated with a Semesan solution. Limitation of space, as well as of time, naturally puts a definite restriction upon the extent of trials designed for the purpose of reliable appraisal of relative resistance or susceptibility. The method finally adopted was to plant 60 seeds of each parental culture, F_1 cross, or F_3 line. These seeds were distributed as follows: Thirty were inoculated with the pathogene and planted immediately in two replicates of 15 seeds each. The remaining 30 were treated with the Semesan solution and also planted in two replicates of 15 seeds each, thus serving as checks. Earlier experiment had shown that very little difference was discernible between the plants from seed planted without any treatment whatsoever and plants from those treated with Semesan. Consequently, it was concluded that

extraneous organisms already located upon the seed or possibly in the sand had little effect in influencing the incidence of disease symptoms on seedlings. Nevertheless, in order to obtain an accurate estimate of the germination and growth potentialities of the lines tested, the check plants were always treated with Semesan before planting.

To expedite the inoculation and planting of the seeds, the following procedure was adopted: The sand in the benches was thoroughly wetted, and holes each 1½ inches deep were stamped in it for the reception of the seeds. For this purpose, a stout board studded with pegs arranged in rows 2 inches apart and an inch between the pegs was employed. Hence the various lines could be planted at a uniform depth and in a regular successive manner. In inoculating, each group of 15 seeds was immersed for a few seconds in a solution containing a mixture of spores and mycelial fragments and immediately planted. The sand was then lightly raked over, so as to cover the holes. The inoculum was prepared by mixing tap water with a pure culture of *Gibberella saubinetii* which had been growing for about 10 days on a moist mixture of wheat and oat grains. The mixture was strained through cheesecloth to remove the grains. Throughout the experiments, an effort was made to maintain the concentration of the inoculum as uniform as possible by so diluting it until about 30–50 spores were visible under the low-power field of the microscope. Planting and treatment of the check plants were carried out in an exactly similar manner, except that a 0.02 per cent solution of Semesan was substituted for the spore suspension.

About 4 weeks after planting, the seedlings reached a stage suitable for note taking. To illustrate the method adopted in this work, an excerpt from the notebook is given below:

Culture number	Greenhouse number	Total number of seedlings	Number stunted	Number not appearing	Vigor	Number free from infection	Number locally infected	Number completely infected
3	A 8	3	2	12	1	.....	.....	15
	B 8	5	2	10	1	.....	.....	15
	C 8	12	.....	3	3			
	D 8	9	.....	6	3			
4	A 9	13	1	2	4	7	2	6
	B 9	12	3	3	3	2	2	11
	C 9	15	.....	.....	5			
	D 9	15	.....	.....	4			

The letters A and B represent the replicates inoculated with Gibberella, while the letters C and D represent these replicates treated with Semesan.

In any line, some plants generally appear, above the ground, to be quite healthy and they remain so. Others reach a height of ½ inch to 2 inches. and then, because of destruction of the subterranean parts by the fungus, begin to wilt and finally become stunted or die. Still others do not appear at all. Therefore, provision is made in the notes for these characters. In obtaining the notes on vigor, an arbitrary scale was set up ranging from 1 to 5. Thus, a line possessing highest vigor, as judged by its general aspect, was designated as 5, one possessing medium vigor as 3, one showing little vigor as 1, and so on. Skill in the allocation of indicative numbers is a matter of experience, which a little practice soon gives.

In estimating the degree of resistance possessed by each line when grown under conditions favoring infection, some criterion becomes necessary. Although an attempt is made to maintain the seedlings of any culture under exactly similar environmental conditions, it is obvious either that such uniformity rarely obtains or, else, the seeds in each selfed line or F_1 cross are not homozygous, for some seedlings remain healthy, while others in the same culture may wilt or die even before emergence. (Figs. 1, 2, 3, and 4). We presuppose that homozygosity exists, so that we must conclude that a state of "balance" exists between the forces contending for the life of each culture. A few cultures are able to withstand wholly the inroads of the fungus; in some slightly varying opportunities for infection lead to the death of some seedlings and the continuance of others. It is therefore reasonable to assume that the higher the degree of resistance, the greater the number of surviving plants in each culture. Consequently, the number of plants appearing healthy above ground was employed as a basis for estimating the degree of resistance. To arrive at this number, the "number stunted" was subtracted from the "total number," in each case. Thus was obtained a number for each replicate, varying from 0 to 15. But it was considered that this number was subject to some correction, due to its being influenced to some extent by the germination capability of each culture. Accordingly, a correction was made on the basis of the germination percentage of the same culture in the Semesan-treated replicates. Thus, for instance, if in these two replicates a total of 25 seedlings emerged out of a possible 30, the number of healthy plants in each of the inoculated replicates was multiplied by 30/25, and the resulting figure used as the final index of resistance. This correction for germination was applied throughout the whole study. If the number allocated to a culture approached 30, then the line was considered resistant; if nearer zero, the line was considered susceptible. A complete range extended between these two extremes.

Admittedly, this scheme is by no means wholly accurate or indeed reliable in making fine distinctions between lines of nearly corresponding

resistance. The number of plants appearing healthy is the resultant not only of genetic potentiality but also of influences such as temperature, moisture, germination capability, and differences in inoculation. These factors all tend to obscure the intrinsic powers of resistance of the various lines, and their easily variable nature is capable of radically influencing the final result.

The notes in the three last columns in the notebook were taken with the intention of finding out to what extent lesions on the subterranean parts influenced the appearance of the subaerial parts. The headings of the columns are self-explanatory. To obtain this information, every plant was dug up, after earlier notes had been taken, and macroscopically inspected to ascertain the extent of the lesions.

EXPERIMENTAL RESULTS

The reliability of the method of experiment as a means of ascertaining inherent resistance to Gibberella saubinetii: Limitation of material compels a compromise between expedition of the experiment and more extensive and perhaps more accurate investigation. As stated in the foregoing section, the method adopted was to grow 15 seeds of each culture in duplicate, in both the inoculated and the check material. In figure 1 the difference between the inoculated and Semesan-treated plots can be seen readily. It will be noted that in each of the two crosses illustrated by figure 1 the F_1 exhibits some degree of resistance, while, of the parents of each cross, only one, viz. culture 43, shows a high degree of resistance. As a means of evaluating the degree of constancy of reaction, the correlation between the two replicates of the inoculated cultures was ascertained. The values correlated were those obtained after the correction had been made for germination percentage. The correlation coefficients were calculated for the parental lines, the F_1 crosses, and for the F_3 lines originating from five different crosses. They are given in table 1.

It will be observed that the correlation coefficient in the case of the selfed lines is negative, is low for the F_1 crosses, and considerably higher in the case of the F_3 lines originating from the crosses 49×50 and 58×60, while in the other three examples 43×46, 43×47, and 64×66, the coefficient is high and significant. There are several reasons for this variance. The F_1 crosses were tested in the greenhouse at a different and earlier period than the F_3 lines, and the average temperature of the soil over this period was about 1° to 2° C. below the average temperature at which the F_3 lines were tested. This was due to faulty placing of the thermocouples, which registered the temperature at a spot constantly higher than the greater part of the sand in the benches. The result was that the number of surviving plants in each replicate was reduced materially. Conse-

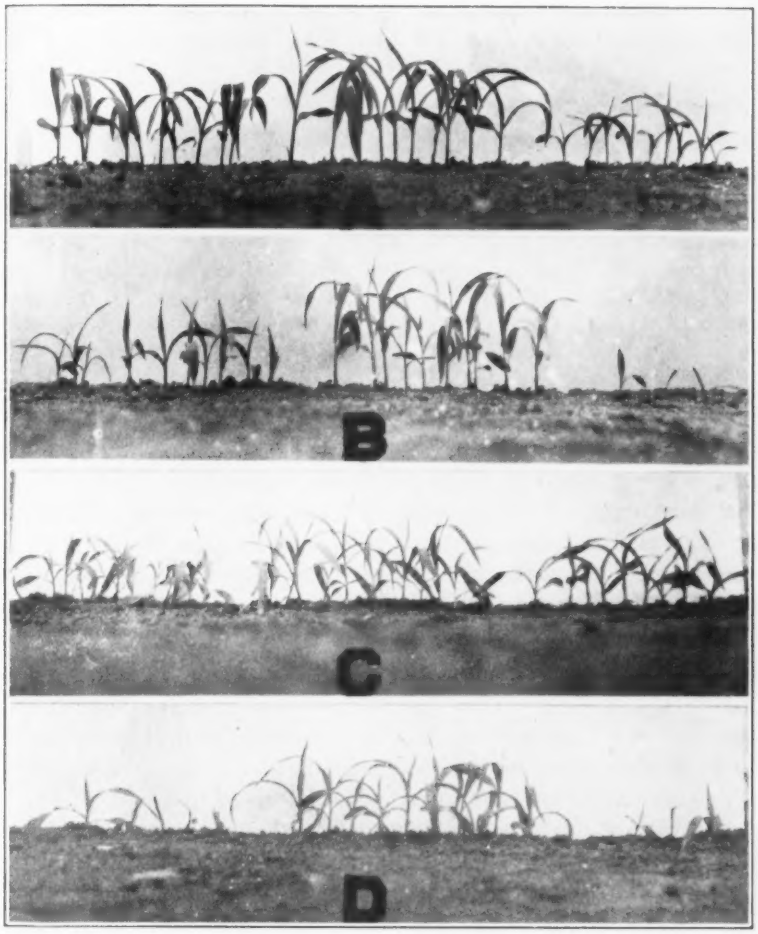

Fig. 1, A. Check plots from Semesan-treated seed under controlled temperatures in the greenhouse. Left, culture 43; right, culture 46; center, culture 43 × culture 46. B. Inoculated plots, seed immersed in an emulsion of spores and mycelium of *Gibberella saubinetii*. From left to right, same cultures as in A. C. Check plot; left, culture 49; right, culture 50; center, culture 49 × culture 50 (compare with Fig. 4). D. Inoculated plot; left, culture 49; right, culture 50; center, culture 49 × culture 50.

quently, the range of values obtained from the selfed lines and F_1 crosses was narrowed considerably in comparison with that from the F_3 lines. This naturally would have the effect of reducing the magnitude of the correlation. In addition the number of plants in each plot (15) is already low enough for a reliable test, and when this number is reduced to 2, 3, 4, or 5, small differences between corresponding plots have a disproportionate effect upon the correlative value. Moreover, on account of the lower tem-

TABLE 1.—*Correlation coefficients between the first and second replicates of selfed lines, F_1 crosses, and F_3 lines when grown from inoculated seed. The letters R, I, S represent "resistant," "intermediate," and "susceptible"*

	Number of individuals in each population	Type of cross	Correlation coefficient
Selfed lines	27	.....	$-.034 \pm .130$
F_1 crosses	100	.....	$.061 \pm .067$
F_3 lines from 43×46	60	R × S	$.608 \pm .055$
F_3 lines from 43×47	90	R × S	$.493 \pm .054$
F_3 lines from 49×50	45	S × I	$.260 \pm .093$
F_3 lines from 58×60	70	S × S	$.203 \pm .077$
F_3 lines from 64×66	31	I × S	$.599 \pm .077$

perature, a few plots failed to show any plants at all, while perhaps 3 or possibly more appeared in their respective duplicates. When the correction for germination was applied the divergence between such plots was further increased, for only one indicative number was affected. In the F_3 lines the average temperature (computed by taking the mean of the temperatures at 4-hour intervals over a period of the first 20 days) was about 15° C. The result was that the survival number in each plot was on the whole higher than in the selfed lines and F_1 crosses, and variations of a few plants did not affect the correlation so strongly. The correlations between the two replicates of the F_3 lines originating from the crosses 49×50 and 58×60 are lower because here, again, the numbers were reduced to some extent on account of the greater susceptibility of these lines in general. It is to be expected also that the highest correlations would be obtained from lines originating from such crosses as 43×46 and 43×47, for here the two parents exhibit a wide difference in reaction, and the range of types obtained from them would be greater.

Another contributory factor towards reducing the correlation between the replicates of all the cultures is the variation in temperature between different localized areas of the greenhouse. This was particularly noticeable when the selfed lines and F_1 crosses were tested, and a special effort was made to reduce it in subsequent trials, with some degree of success.

These results serve to emphasize the necessity in critical investigation of maintaining not only a suitable but a uniformly distributed temperature in the greenhouse over the period of trial. Increase in number of replicates, in so far as this is compatible with space, would also be conducive to greater accuracy. It may be said, then, that with such improvements, the method of experimentation could be considered sufficiently reliable in future investigation.

The relation between the reaction of parents and that of F_1 crosses between them: In order that environmental conditions under which parental lines and F_1 crosses were grown might be as similar as possible, both parents and crosses were grown in duplicate at the same time and in the same greenhouse. According to the method previously described each line was allotted a number indicating its reaction. Thus the numbers appearing healthy in each of the two plats were corrected on the basis of the germination in the Semesan-treated material, and the two numbers so obtained added together to give the final index.

Realizing that this index can be greatly influenced by small divergences in temperature, it was considered advisable not to classify the individual

TABLE 2.—*Reaction of selfed parents and of F_1 crosses to Gibberella saubinetii. The reaction of each culture is represented by an index number. These numbers range from 0–30 and increase with apparent resistance. Each index is given as the nearest whole number to that actually calculated.*

Cross	Reaction of parents	Reaction of F_1	Cross	Reaction of Parents	Reaction of F_1	Cross	Reaction of parents	Reaction of F_1
41×42	5 and 6	6	46×47	0 and 0	16	55×56	5 and 0	14
41×43	5 " 11	12	46×48	0 " 9	7	55×57	5 " 6	10
41×44	5 " 3	9	46×49	0 " 2	11	55×58	5 " 0	10
41×45	5 " 9	9	46×50	0 " 5	1	55×60	5 " 3	4
41×46	5 " 0	2	47×48	0 " 9	9	56×57	0 " 6	8
41×47	5 " 0	14	47×49	0 " 2	2	56×58	0 " 0	5
41×48	5 " 9	10	47×50	0 " 5	12	56×60	0 " 3	1
41×49	5 " 2	10	48×49	9 " 2	11	57×58	6 " 0	1
41×50	5 " 5	11	48×50	9 " 5	5	57×60	6 " 3	1
42×43	6 " 11	15	49×50	2 " 5	12	58×60	0 " 3	2
42×44	6 " 3	2	51×52	7 " 5	7	61×62	0 " 6	7
42×45	6 " 9	13	51×53	7 " 9	21	61×63	0 " 2	9
42×46	6 " 0	14	51×54	7 " 4	11	61×65	0 " 4	2
42×47	6 " 0	5	51×55	7 " 5	10	61×66	0 " 0	12
42×48	6 " 9	1	51×56	7 " 0	12	61×68	0 " 4	0
42×49	6 " 2	4	51×57	7 " 6	12	62×63	6 " 2	16
42×50	6 " 5	9	51×58	7 " 0	10	62×64	6 " 6	4
43×44	11 " 3	25	51×60	7 " 3	30	62×65	6 " 4	11
43×45	11 " 9	7	52×53	5 " 9	13	62×66	6 " 0	8
43×46	11 " 0	8	52×54	5 " 4	23	62×67	6 " 1	14
43×47	11 " 0	5	52×55	5 " 5	19	62×68	6 " 4	12
43×48	11 " 9	9	52×56	5 " 0	12	63×64	2 " 6	20
43×49	11 " 2	19	52×57	5 " 6	16	63×65	2 " 4	10
43×50	11 " 5	12	52×58	5 " 0	5	63×66	2 " 0	4
44×45	3 " 9	10	52×60	5 " 3	12	63×68	2 " 4	13
44×46	3 " 0	4	53×54	9 " 4	18	64×65	6 " 4	16
44×47	3 " 0	6	53×55	9 " 5	13	64×66	6 " 0	9
44×48	3 " 9	5	53×56	9 " 0	18	64×67	6 " 1	0
44×49	3 " 2	13	53×57	9 " 6	10	64×68	6 " 4	4
44×50	3 " 5	15	53×58	9 " 0	14	65×66	4 " 0	2
45×46	9 " 0	2	53×60	9 " 3	15	65×67	4 " 1	9
45×47	9 " 0	10	54×55	4 " 5	9	65×68	4 " 4	24
45×48	9 " 9	5	54×56	4 " 0	15	66×67	0 " 1	14
45×49	9 " 2	5	54×57	4 " 6	15	66×68	0 " 4	21
45×50	9 " 5	8	54×60	4 " 3	14	67×68	1 " 4	8

lines as resistant or susceptible but to present the results directly. This is done in table 2. The corrected indices are listed as the nearest whole number to those actually computed. It will be seen that the indices of the selfed parents never approach the magnitude of some of the crosses. This in itself indicates the quantitative rather than the qualitative character of the inheritance and suggests that the genetic factors involved are multiple. The appearance of a cross showing a high index from two parents of low indices further substantiates this hypothesis, and such cases may indicate a transgressive inheritance.

It was of interest to calculate the correlations between reaction of the low-index parents and the crosses, between the high-index parents and the crosses, and between the average of the parents and the crosses. These correlation coefficients are as follows:

Between low-index parents and crosses	$.191 \pm .063$
Between high-index parents and crosses	$.114 \pm .065$
Between average index of parents and crosses	$.110 \pm .065$

These coefficients are too low to allow any definite conclusions being drawn from them, but it would seem that in this trial the low-index parent was somewhat more strongly correlated with the reaction of the F_1 cross than was the high-index parent. The fact that most of the parental lines are not remarkable for their resistance might account for this. The low correlation between the average index of the parents and that of the crosses is also scant evidence that a transgressive inheritance is operative over the crosses as a whole. The little understood "incompatibility" between certain selfed lines on crossing may possibly reduce the correlation. It is not suggested that there is a cause and effect relation between vigor and resistance, but it is possible that when two parents, themselves possessing little intrinsic resistance, unite to give a vigorous hybrid, this hybrid may be able to produce adventitious roots at an early date and thus "avoid" extinction. It is certain, however, that all cultures with well-developed adventitious roots showed a high index, though the converse was not always true, e.g., in culture 53, variety Rustler, and most crosses involving it. It might be possible to separate partially the effects of intrinsic resistance and early development of roots by using such cultures in future experiment. Cultures with strong adventitious roots sometimes exhibited lesions of considerable extent upon the mesocotyl and other embryonic structures, and sometimes they did not. The subterranean parts of all plants inoculated with the fungus were examined (about 12,000 plants in all), and in no case was any culture found free of lesions in all its constituent plants; even the most resistant culture showed only half of its plants entirely free from visible symptoms. While many lines seemingly succumbed easily to

the invasion of the parasite, some few appeared to offer resistance to its advance, in that the lesions developed on the mesocotyl were quite local, with definitely defined margins. (Cultures 43×49, 44×49.) Possibly these local lesions would have developed more extensively, but their appearance at the same stage of development on numerous plants of the same culture indicates that a definite resistance was being offered to the advance of the parasite.

There is no evidence in support of the statement of Dickson and Holbert (4) that susceptibility is dominant over resistance. Rather is it probable that the inheritance is quantitative, conditioned by multiple factors. The appearance of apparently resistant crosses in some few cases from parents of lower index indicates a possibility of obtaining resistant lines from susceptible strains.

Examination of F_3 lines from selected F_1 crosses: In order to gain a more extensive knowledge of the inheritance involved, certain crosses which exhibited marked differences between their respective parents were grown in the field in 1928, and the selfed progeny of each cross retained. Ears were selfed at random on F_2 plants grown in 1929. Thus, in 1929, a number of F_3 lines were available, 60 seeds from each of which were grown in the winter of 1929–30. The same procedure of planting in the greenhouse was adopted as for the parental lines and F_1 crosses and the same correction for germination made on the basis of the check plots. The crosses that were selected and the number of F_3 lines available from them in 1929 are as follows:

Cross	Supposed nature of cross	Number of lines available
43×46	R×S	60
43×47	R×S	90
49×50	S×I	45
58×60	S×S	70
64×66	I×S	31

Had the reaction of the F_1 cultures been known at the time of the selection of these crosses, others would doubtless have been added or substituted. For instance, 43×44 (R×S) was exceptionally resistant when tested in the F_1; 62×68 (S×S) also proved resistant in the F_1. This remains an avenue of investigation yet to be followed.

In order that a comparable basis for estimation of relative resistance of parental lines, F_1 crosses, and F_3 cultures might be available, the parental

lines and F_1 crosses that gave rise to the F_3 cultures were tested again at the same time. It has been stated already that this experimental series was carried out at a temperature of about 1° to 2° higher than that obtaining when the parents and F_1 crosses were first tested. Table 4 gives the results obtained, and by comparison with similar cultures of the parents and crosses in table 2 the influence of the difference in temperature is well evidenced. For convenience the results from the two trials are contrasted in table 3.

TABLE 3.—*Comparison between indices obtained from different cultures when grown at a temperature difference of 1–2° C. The first trial was at about an average temperature of 14° C., the second at about 15° C.*

Culture	Index obtained from 1st trial	Index obtained from 2nd trial
43	11	21
46	0	2
47	0	2
49	2	5
50	5	7
58	0	0
60	3	0
64	6	13
66	0	9
F_1 43 × 46	8	27
F_1 43 × 47	5	—[a]
F_1 49 × 50	12	29
F_1 58 × 60	2	8
F_1 64 × 66	9	27

[a] Seed not available.

It is obvious from the comparison that the indices of nearly all the cultures are increased as a direct effect of the rise in temperature. The increase is by no means proportionate, the crosses showing the greatest amount, while cultures 58 and 60 exhibit no increase whatever. It would seem that different cultures react differently to the same increase in temperature. Consequently, the variance between different cultures is increased at the higher temperature (15° C.) and this naturally is advantageous when the number of plants in each culture is restricted.

Table 5 is an analysis of the data presented in table 4. The F_3 lines are here arranged in 10 categories of descending degree of resistance as represented by the indices. In each category appears the number of F_3 lines of a similar index.

TABLE 4.—*Reaction of parents, F_1 crosses and F_2 lines originating from them, to Gibberella saubinetii. The reaction of each culture is represented by an index number. These numbers range from 0–30, and increase with the apparent resistance. Each index is given as the nearest whole number to that actually calculated*

Line	Index	Line	Index	Line	Index	Line	Index
43	21	40	19	33	29	88	8
46	2	41	14	34	23	89	9
47	2	42	4	35	23	90	5
49	5	43	23	36	26		
50	7	44	10	37	28	F_2 49 x 50	
58	0	45	7	38	19	1	11
60	0	46	12	39	30	2	8
64	13	47	18	40	16	3	10
66	9	48	27	41	21	4	11
F_1 43 x 46	27	49	23	42	25	5	10
F_1 43 x 47	[a]	50	15	43	29	6	16
F_1 49 x 50	29	51	12	44	18	7	5
F_1 58 x 60	8	52	15	45	20	8	16
F_1 64 x 66	27	53	13	46	19	9	2
		54	26	47	25	10	13
F_2 43 x 46		55	2	48	6	11	11
1	11	56	21	49	20	12	13
2	16	57	10	50	17	13	15
3	6	58	16	51	20	14	21
4	21	59	10	52	13	15	10
5	14	60	18	53	10	16	19
6	7			54	10	17	16
7	20	F_2 43 x 47		55	27	18	15
8	15	1	20	56	6	19	19
9	22	2	10	57	13	20	13
10	13	3	6	58	9	21	12
11	7	4	2	59	24	22	12
12	10	5	20	60	4	23	12
13	28	6	15	61	15	24	15
14	19	7	10	62	18	25	20
15	23	8	12	63	16	26	15
16	28	9	7	64	24	27	17
17	14	10	9	65	18	28	3
18	26	11	5	66	24	29	16
19	22	12	21	67	25	30	24
20	12	13	18	68	20	31	16
21	11	14	14	69	17	32	24
22	18	15	22	70	28	33	13
23	28	16	9	71	13	34	14
24	23	17	17	72	17	35	15
25	29	18	14	73	9	36	18
26	14	19	16	74	16	37	11
27	21	20	22	75	12	38	22
28	13	21	13	76	20	39	17
29	21	22	19	77	15	40	20
30	13	23	15	78	18	41	12
31	17	24	18	79	10	42	26
32	17	25	22	80	17	43	9
33	16	26	25	81	16	44	11
34	17	27	24	82	26	45	9
35	23	28	28	83	7		
36	10	29	18	84	22	F_2 58 x 60	
37	11	30	18	85	18	1	5
38	21	31	22	86	9	2	4
39	23	32	20	87	27	3	4

[a] Seed not available.

TABLE 4.—(*Continued*)

Line	Index	Line	Index	Line	Index	Line	Index
F_3 58 × 60		29	10	55	5	9	8
4	7	30	16	56	3	10	10
5	9	31	7	57	4	11	7
6	0	32	14	58	7	12	11
7	4	33	5	59	4	13	9
8	1	34	2	60	2	14	7
9	3	35	11	61	5	15	24
10	2	36	8	62	1	16	21
11	13	37	5	63	4	17	13
12	1	38	4	64	6	18	15
13	14	39	10	65	10	19	20
14	7	40	8	66	4	20	2
15	11	41	11	67	3	21	9
16	13	42	1	68	4	22	10
17	15	43	1	69	2	23	11
18	14	44	6	70	1	24	3
19	5	45	6			25	18
20	9	46	1	F_3 64 x 66		26	27
21	6	47	2	1	13	27	14
22	9	48	10	2	5	28	10
23	5	49	7	3	9	29	9
24	13	50	9	4	21	30	14
25	4	51	3	5	4	31	11
26	8	52	2	6	0		
27	4	53	1	7	8		
28	7	54	1	8	12		

Examination of this table will show that the distribution of the numbers in each class varies according to the type of original cross from which the F_3 lines originated and is in accordance with what might be expected from a Mendelian segregation of quantitative factors. Where a wide disparity exists between the degree of resistance of the original parents, as in 43 × 46 and 43 × 47, lines appear in the F_3 which also exhibit very considerable difference. Where the disparity between the parents is not so marked, but where they still possess an intermediate degree of resistance, as in 49 × 50 and 64 × 66, the F_3 lines also exhibit differences, but these are not quite so wide as in the two former cases. This may be partly, but not wholly, ascribed to the fewer individuals studied in the two latter examples. In the fifth cross, 58 × 60, where both parents are quite susceptible to the disease, no very resistant F_3 line appears, and indeed the modal class occurs at the lower end of the scale.

The distribution of these F_3 lines from each cross has been represented graphically in figures 2, 3, 4, 5 and 6. The solid line represents the curve obtained from the actual populations, while the dotted line represents the curve drawn when the populations are considered as 100 in each case. The position of the modal class will be seen to be nearer the lower end of the curve in all except one of the examples, viz, 43 × 47. The location of this

TABLE 5.—*Analysis of the reaction of parents, F_1 crosses, and F_3 lines, to Gibberella saubinetii. Indices are used as in tables 2 and 4. The F_3 lines are arranged in 10 categories of descending degree of resistance, i.e., in class frequencies*

Variety	Cross	Character of cross	Reaction of F_1	F_3 lines arranged in categories according to their reaction										Total number
				30–28	27–25	24–22	21–19	18–16	15–13	12–10	9–7	6–4	3–0	
Minn. No. 13	43 × 46	21 × 2	27	4	3	8	8	9	11	11	3	2	1	60
Minn. No. 13	43 × 47	21 × 2	[a]	6	8	11	13	19	10	7	8	7	1	90
Minn. No. 13	49 × 50	5 × 7	29		1	3	5	8	10	12	3	1	2	45
Rustler	58 × 60	0 × 0	8					1	7	8	12	22	20	70
N. W. Dent	64 × 66	13 × 9	27		1	1	3	1	5	7	8	2	3	31

[a] Seed not available.

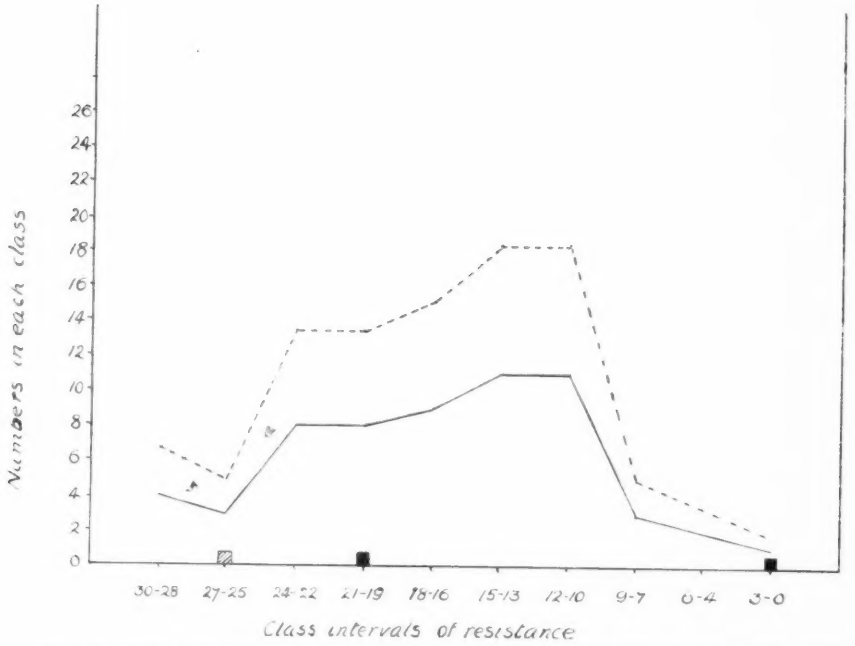

FIG. 2. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 43 × 46. The solid line represents the actual numbers (60), while the dotted line represents the population on a basis of 100. The two black squares indicate the classes into which the parents fall, and the shaded square the class into which the F_1 cross falls.

modal class should indicate which cross combines the greatest number of factors tending to increase resistance.

Where quantitative inheritance has been studied, it has been usual to find that parental types appear in later generations. In this study, it will be noticed that if the indices are interpreted literally, not only are the apparent parental types recovered, but segregates appear far in excess of the parents in resistance. The extent to which the parental lines would have varied had they been tested as extensively as the F_3 hybrids is unknown. Selfed lines do not exhibit the degree of resistance of the majority of crosses, and the indices of the selfed lines are to be regarded as merely indicating their *relative* resistance and are not to be compared directly with the indices of later hybrid generations. It would seem that resistance to *Gibberella saubinetii* is analogous to vigor in maize in that continued selfing isolates a range of segregates which do not approach the degree of resistance of the F_1.

In the first two crosses of Minnesota No. 13, viz, 43 × 46 and 43 × 47, a comparatively high proportion of resistant lines appears in the F_3 as well

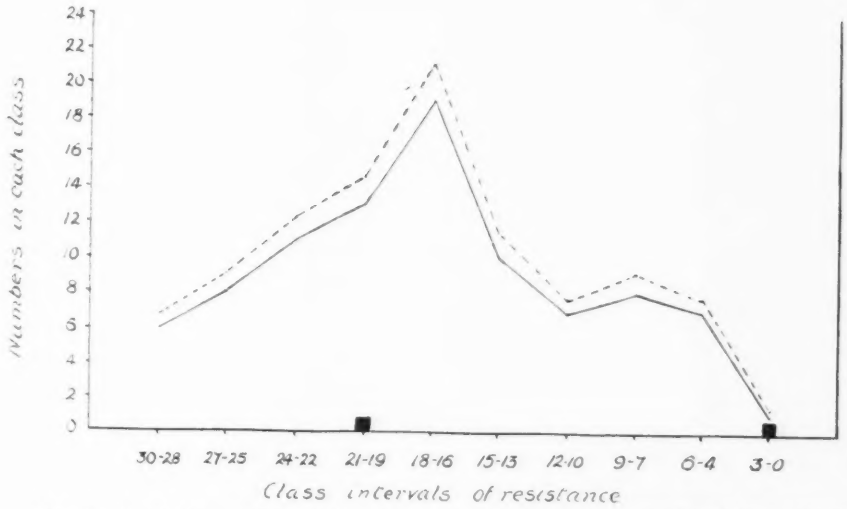

FIG. 3. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 43 × 47. The solid line represents the actual numbers (90), while the dotted line represents the population on a basis of 100. The two black squares indicate the classes into which the parents fall. In this case, seed of the F_1 was not available for testing.

as a number of quite susceptible lines. The uniformity of reaction of the plants composing each of these diverging lines points to their approach towards homozygosity in respect to disease resistance. Many of the lines of intermediate index also appeared uniform within themselves, but quite a few displayed marked differences in their individual constituent plants, from which it was inferred that segregation was in progress within the line. Such appearance of resistant and susceptible strains in a generation as early as F_3 might possibly be interpreted as indicative that the factors conditioning resistance are not numerous. But, recalling the easily variable nature of the reaction, such a postulate should be advanced with caution.

Observations on vigor and resistance: If resistance to root rot in the seedling stage is connected in some way with growth factors, then it might be supposed that vigor and resistance of seedlings would be correlated in some degree. Peterson (9) has denied any such relationship, and it was a matter of common observation during the present investigation to find, as did Peterson, strains that displayed much vigor but little resistance. Holbert *et al.* (7) have observed that seeds selected from diseased mother plants produce plants deficient in early vigor, even though infection of such seeds could not be detected by a germination test. There was opportunity to correlate mathematically the vigor of all the F_3 lines in the check bench with their respective indices of resistance in the inoculated material. The

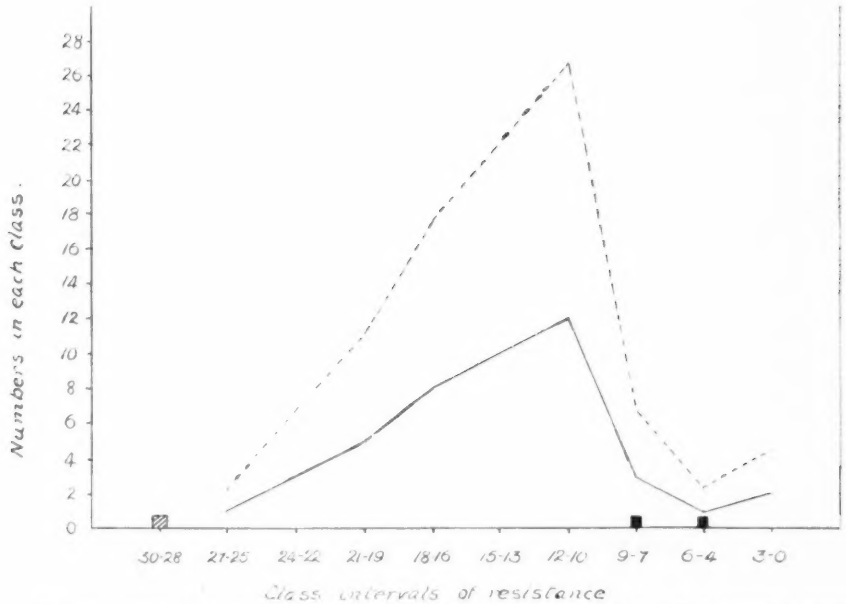

FIG. 4. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 49 × 50. The solid line represents the actual numbers (45), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

criterion of vigor was a scale of 1 to 5, in which the lower numbers represented low vigor, the high numbers greater vigor. Each plot in the check material was thus appraised, and, by adding together the two figures obtained from the corresponding plots, a range of values from about 3 to 10 was procured. These values were then correlated directly with the indices of reaction obtained from the inoculated cultures. Correlation coefficients were calculated for each of the five groups of F_3 lines, and these are presented below in table 6.

TABLE 6.—*Correlation between vigor of growth of seedlings in the check plots and reaction to Gibberella saubinetii in inoculated bench plots of F_3 cultures*

Variety and culture	Reaction of original parents	Average index of vigor	Correlation coefficients
Minn. 13 F_3 43 × 46	21 and 2	6.52	.199 ± .084
Minn. 13 F_3 43 × 47	21 " 2	6.27	.519 ± .052
Minn. 13 F_3 49 × 50	5 " 7	7.89	.155 ± .098
Rustler F_3 58 × 60	0 " 0	7.81	.107 ± .080
N.W. Dent F_3 64 × 66	13 " 9	7.45	.382 ± .104

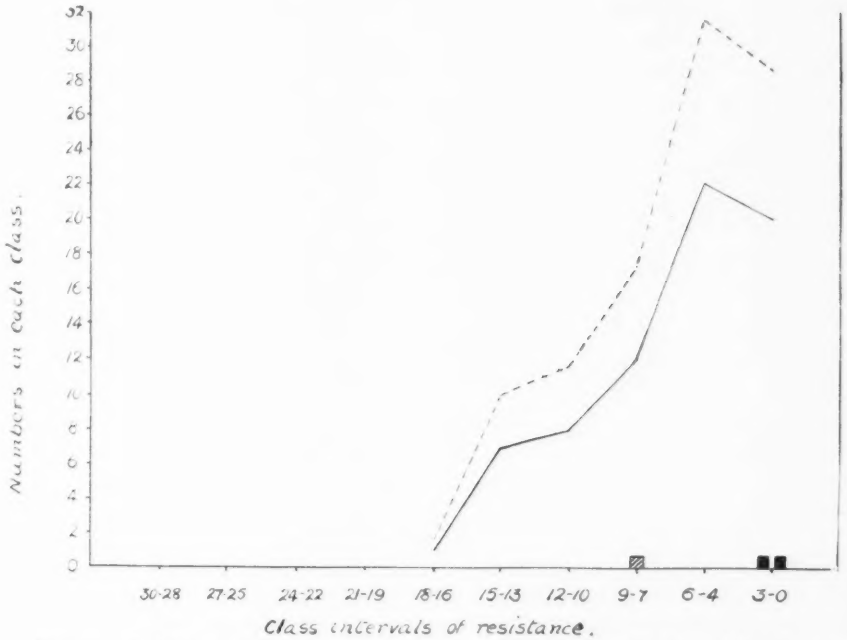

FIG. 5. Graphical representation of the relative distribution of resistance among the F_3 segregates from the original cross 58×60. The solid line represents the actual numbers (70), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

It is natural that in lines possessing little resistance correlation between vigor and resistance would be small. This is made apparent by the above table and exemplified by the two groups of F_3 lines from 49×50 and 58×60. On the other hand, these groups whose lines possess some degree of resistance tend to show significant correlations between vigor and resistance, *e.g.*, 43×46, 43×47, and 64×66. The groups are selected populations, and it is in accordance with expectation that such a result is found. Indeed, when all five groups were combined, the coefficient of correlation was found to be $-.002 \pm .039$, and, if the experiment were interpreted on this result, it would seem as if no correlation whatever existed. But to include such groups as 58×60 and 49×50 would not be logical, for factors for resistance are almost absent in them and no opportunity for variability exists.

While we are justified therefore in concluding that a definite correlation exists between vigor and resistance, the relation is by no means sufficiently constant to permit the practice of selection for resistance on the basis of seedling vigor.

The correlation between seedling reaction in the greenhouse to Gibberella saubinetii and yield in the field: If greenhouse testing of seedlings is to be

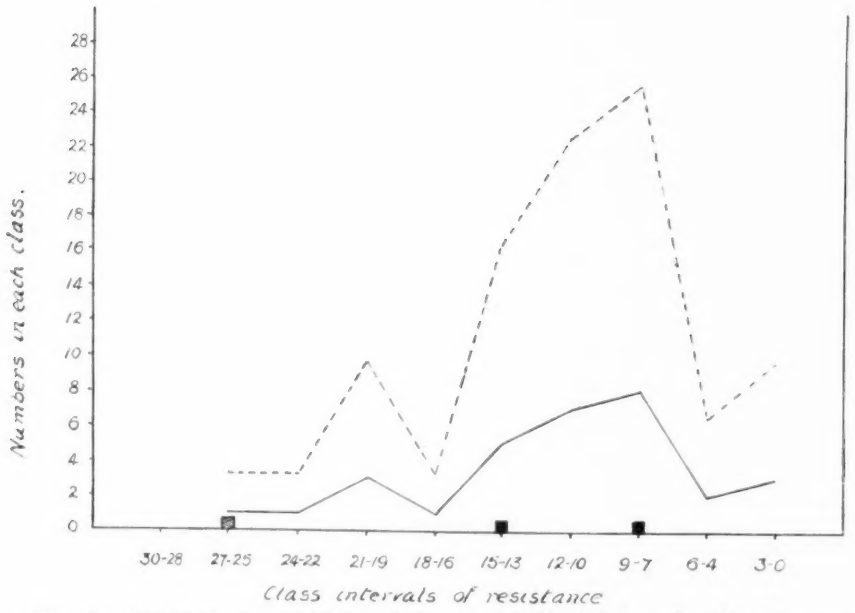

FIG. 6. Graphical representation of the relative distribution of resistance among the F_2 segregates from the original cross 64 × 66. The solid line represents the actual numbers (31), while the dotted line represents the population on a basis of 100. The black and shaded squares are used in the same way as in figure 2.

employed as a means of discriminating between resistant and susceptible lines, then it becomes of importance to know whether resistant lines thus delimited will show actual advantage over others when grown in the field. In short, we wish to know what advantage, if any, accrues to the breeding of corn which shows seedling resistance. Do resistant seedlings generally give higher yields, and do susceptible seedlings as a rule give lower yields? If this is the case, then we could expect to find a positive correlation between greenhouse resistance and yield in the field.

Holbert *et al.* (7) have found that "corn populations grown from seed . . . susceptible to the root and stalk rots produced a much lower yield of sound marketable corn . . . than corn grown from seed relatively . . . resistant to infection." On the other hand, Kiesselbach (8) has reported that "selection for freedom from root-rot disease by the germinator test does not increase grain production under the conditions of the experiment."

Field data upon yield taken in 1927 and 1928 were available from most of the crosses which had been tested for their reaction to disease in the greenhouse. These yields were computed from two systematically distributed replicates, each plot consisting of 12 hills, and bordered on both sides by the normal variety. Each hill was originally planted with 5 seeds.

and later thinned to 3. Only 2 and 3 plant hills were harvested, and these only when bordered on all 4 sides by 2 or 3 plant hills. Due allowance was made for the number of hills harvested, and the corrected yield expressed in percentage of the normal variety. These yields were computed primarily for the purpose of comparing the inherent yielding capacity of the F_1 crosses made at University Farm in connection with maize-breeding investigations.

It will be seen that with this method of computing yields, much of the influence of seedling injury upon the final yield is eliminated. For, in the first place, thinning to 3 seedlings is likely to cause retention of those seedlings which have escaped thorough infection, and in the second place, harvesting 2 and 3 plant hills would tend to eliminate the effect of those plants which died after thinning.

Consequently, by correlating directly the indices expressing resistance with the yields of the respective crosses, an opportunity to ascertain the effect of fungous invasion of the mature plants upon the yield of the crosses was afforded. In table 7 are given these correlations, with the yields of the separate varieties in 1927 and 1928 and also with the average yields of the same varieties for the 2 years. In addition, similar correlations are given when all 3 varieties are considered as one.

TABLE 7.—*Correlation coefficients between seedling reaction in the greenhouse and yield in the field*

Variety	1927	1928	Average of 1927 and 1928
Minn. No. 13	.317 ± .126	−.086 ± .107	.029 ± .141
Rustler	−.099 ± .138	.079 ± .116	.102 ± .139
N.W. Dent	.345 ± .127	.030 ± .147	.169 ± .155
Minn. No. 13, Rustler, N.W. Dent	.219 ± .077	−.024 ± .070	.103 ± .083

It will be seen from table 7 that the figures are contradictory. Only in 1927 do positive correlations of any magnitude appear, and, even in these, the probable error is so large (as in all cases) as to remove significance from the results. Even negative correlations appear in three cases. The only statement that can be made is that no correlation that is at all significant emerges from the data.

CONCLUSIONS AND SUMMARY

1. The investigation of the inheritance of maize to root-rotting organisms is fundamental to any rational method of producing resistant varieties. Owing to the nature of the disease, however, such investigation meets with experimental difficulties, and it was the purpose of this study not only to

attempt to elucidate the inheritance but to examine the methods employed and to appraise their dependability.

2. It is emphasized that uniformity of environmental influences, particularly temperature, is a desideratum if constancy of reaction to the disease is to be sought in any culture of maize. Not only should the temperature over the first 3 weeks of the experimental period be constant but equal distribution of temperature over the greenhouse should be assured. Especially is this latter condition to be fulfilled if dependable estimation of relative resistance is desired. The most suitable soil temperature at which to conduct the work was found to be about 15° C. When such a temperature was maintained, the correlation between replicates was sufficiently high (considering the small numbers used) to warrant drawing conclusions from the data.

3. Crosses of susceptible and resistant parents gave conflicting results in the F_1 generation. Sometimes a highly resistant hybrid resulted, and at other times the hybrid proved quite susceptible. Occasionally resistant hybrids appeared from parents possessing an intermediate degree of resistance, but in no case did very resistant progeny appear from parents of extreme susceptibility, although such crosses sometimes proved to be of intermediate resistance. It is impossible to state that resistance is either dominant or recessive to susceptibility. Rather is it more logical to assume that the inheritance is quantitative in nature and conditioned by multiple factors.

4. Examination of the F_3 progeny of 5 selected crosses gave further evidence of a quantitative inheritance. A definite segregation of F_3 lines occurred in conformity with a Mendelian explanation of such inheritance. Where the parental lines differed widely in reaction, highly resistant and susceptible lines appeared in the F_3, in sufficient proportion to suggest tentatively that perhaps relatively few factors are involved for intrinsic resistance.

5. In F_3 lines obtained from parents differing widely in resistance, a definite and significant correlation was found to exist between seedling vigor and resistance, though the relation is not sufficiently constant to warrant selection for resistance on the basis of seedling vigor.

6. No significant correlation was found to exist between seedling reaction in the greenhouse and yield in the field as affected by fungous invasion of mature plants.

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BROWN SPOT OF TOBACCO CAUSED BY ALTERNARIA LONGIPES (E. & E.), N. COMB.

W. B. TISDALE AND R. F. WADKINS[1]

INTRODUCTION

The tobacco plant, whether grown for flue-cured, sun-filler, or shade-wrappers, is subject to leaf spotting caused by several different parasitic organisms throughout the Florida-Georgia tobacco district. Most of this leaf spotting, with the exception of wild fire and black fire, which are caused by bacterial organisms, may be attributed to different fungi. The most common fungi which may cause leaf spotting of tobacco of any consequence in Florida are *Phyllosticta nicotiana* E. & E., *Cercospora nicotiana* E. & E., and the species of Alternaria herein considered.

Because of the fact that leaves of bright tobacco are left on the stalks much longer than any other type of tobacco grown in this district before harvesting and therefore reach a more advanced stage of maturity, conditions for the development of brown leaf spot are more ideal. Hence, the brown leaf-spot disease is one that most commonly concerns the growers of bright tobacco. The information reported herein has been obtained from investigations of the disease on that crop, as the disease has been of little importance on cigar-wrapper tobacco.

THE DISEASE

In 1892 two species of Macrosporium were reported on tobacco from North Carolina (3). Since that year, various reports from Connecticut, Massachusetts, Missouri, North Carolina, Ohio, and Pennsylvania (1)[2,3,4] have been made, but in each case the disease and the organisms concerned seem to have been given very little attention. During the seasons of 1923 and 1924, Thomas reported[5] a species of Alternaria causing brown spots on tobacco leaves in Ohio. Isolations were made, but, as the disease was considered of minor importance, the cultures were discarded.[6]

[1] Formerly laboratory assistant, Tobacco Experiment Station, Quincy, Fla.

[2] Fant, G. W. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 45: 127. 1926. (Mimeographed.)

[3] Thomas, R. C. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 26: 143. 1923. (Mimeographed.)

[4] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 34: 234. 1924. (Mimeographed.)

[5] *Op. cit.* (see footnote 4).

[6] Information contained in a letter from R. C. Thomas, dated February 5, 1929.

When the brown-spot disease first made its appearance in Florida is not known, but its occurrence did not become conspicuous until 1924, when flue-cured tobacco was introduced and grown on an extensive commercial scale (6). However, it is probable that the organism causing the disease occurred unobserved on cigar tobacco or on some other host plant prior to that time. Brown leaf spot usually makes its appearance each year during the latter half of July, occurring first on the sand leaves and spreading progressively upward to the top of the plant. The sand leaves are usually of poor quality and many growers leave them in the field, either on the stalk or on the ground. This practice may prove to be a source of later infection for the leaves higher up the stalk.

The first records of the disease in Florida were made by W. B. Tisdale, who observed it on flue-cured tobacco on a plantation in the southern part of Gadsden County, on July 24, 1925. Later, during the same growing season, the disease was observed in several widely separated areas. The disease now appears generally distributed over the entire Florida-Georgia district.

ECONOMIC IMPORTANCE

The total loss sustained each year by the growers of flue-cured tobacco as a result of brown spot depends largely upon the weather conditions and the cultural methods employed. Should premature ripening of the leaves occur as a result of insufficient moisture or from severe root-knot infection, the loss may be greater than under normal conditions. Also, improper cultural methods, such as late, deep plowing near the plants or delayed priming operations, may result in greater losses from the disease. However, the loss from brown spot may be considered somewhat minor as compared with the more important diseases of tobacco, such as wild fire, black fire, and black shank, when these occur. This is especially true under normal conditions when the leaves are harvested at the proper stage of ripeness. A crop of tobacco, severely spotted, will bring a much lower price than a crop that is comparatively free of spotting. The exact reduction in price per pound because of brown spot is not definitely known. If the infection is slight, the buyer often considers it an indication of ripe tobacco. However, it has been observed that a reduction in price was made on the Quincy market during the past 2 years for tobacco severely damaged from a leaf spotting caused by *Cercospora nicotiana* E. & E. and Alternaria. Therefore, it would be largely guesswork to attempt to give an estimate of the loss due to brown spot alone, since it develops under similar conditions and often simultaneously with the former organism. In 1925 Boyd[7]

[7] Boyd, O. C. U. S. Dept. Agr., Bur. Plant Indust., Plant Disease Rptr. Sup. 45: 127. 1926. (Mimeographed.)

reported a loss of 5 per cent of flue-cured tobacco in Georgia, which he attributed to a species of Macrosporium. In 1926, he again reported[8] the same disease in the South Georgia district. Boyd[9, 10] also has estimated the losses produced by *Alternaria* sp. for 1927 and 1928. Tisdale[11] reported the disease in Gadsden County, Florida, in 1927, but stated that buyers failed to make a reduction in price because of the disease except in cases of severe infection. It is difficult, therefore, to estimate the losses each year due to the Alternaria organism alone.

SYMPTOMS

The spots appear first on the lower leaves and enlarge rapidly. They often coalesce, thus rendering worthless the entire leaf area. As the leaves mature successively upward, spotting follows, and in severe cases, lesions appear on the stalks.

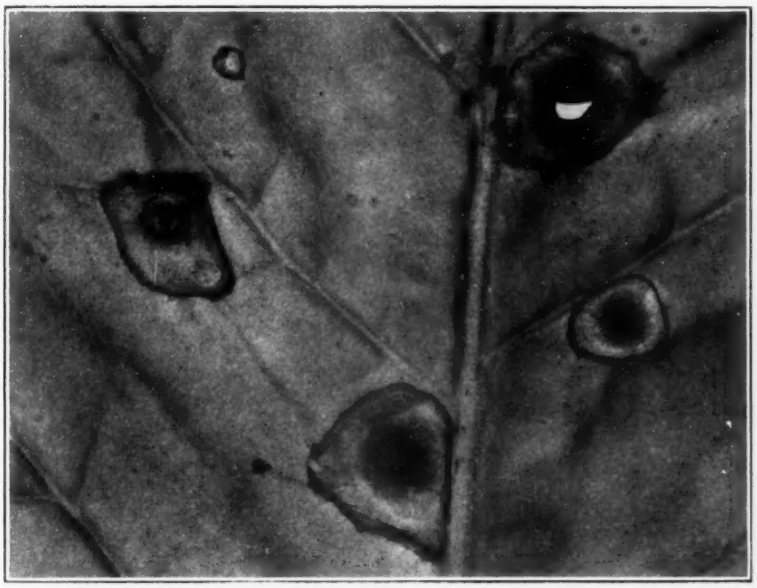

FIG. 1. Typical spots on White Stem Orinoco tobacco leaf produced by *Alternaria longipes* under field conditions. (Slightly enlarged.)

[8] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 54: 316. 1926. (Mimeographed.)

[9] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. Sup. 61: 287. 1928. (Mimeographed.)

[10] ———. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. 12: 115. 1928. (Mimeographed.)

[11] Tisdale, W. B. U. S. Dept. Agr., Bur. Plant Indus., Plant Disease Rptr. 11: 138. 1927. (Mimeographed.)

The spots appear first as small, water-soaked areas which gradually enlarge, generally in a circular shape, unless checked on one side by the midrib or a prominent lateral vein. As the centers of the spots die and wither, they become sunken on both surfaces. The line of demarcation between the diseased and healthy tissues, under humid conditions, is not distinct, although, during dry periods, it becomes very pronounced. If damp weather should prevail over a prolonged period, there may be a halo margin or, in advance stages of the disease, varying blended shades from green to brown. The spots, under favorable conditions, may reach 1.5 to 2.5 cm. in diameter and may show a faint target-board effect or concentric rings (Fig. 1).

Lesions on the petiole are elongated and depressed and develop more slowly than those on the leaf blade. The lesions on the stalk may be numerous, somewhat sunken, and may finally girdle the plant. However, invasion never extends deeper than the cambium, and usually not that deep. Stem lesions usually do not produce conidiophores and conidia until the plant begins to decline.

Host Range

The information gained with reference to the host range of this particular fungus has been mostly from observations made in the fields at various times during the growing seasons of 1927 and 1928. Tobacco only has been observed to be affected with this organism. Several species of Alternaria have been isolated from various plants but, in each instance, the organism isolated proved nonpathogenic to tobacco.

Inoculation experiments under favorable temperature and humidity conditions with white potatoes, tomatoes, and peppers growing in pots and flats gave negative results.

Susceptibility of Different Varieties of Tobacco

In a test plot, where 24 varieties of bright tobacco were grown, progress of the disease was observed at intervals throughout the growing season. None of the leaves were harvested from the plot and the first leaves to become infected were left on the stalks. As the season progressed, infection developed higher up the stalks. It was observed that the sand leaves were the first to show the disease and by the time the seed pods were ready to be harvested nearly the entire leaf area of all varieties had become involved, with no indication of varietal resistance. The stalks also had numerous lesions throughout the entire length.

During the growing season of 1928, a small plot of ground near the laboratory was planted to the 10 following varieties of bright tobacco: Adcock, Big Stem Orinoco, Bonanza, Cash, Crutcher, Improved Warne, Improved

White Stem Orinoco, McAdoo, Narrow Leaf Orinoco, and Yellow Pryor. On July 9th a few of the lowest leaves of each variety were affected with brown spot, and by July 30th the entire leaf area had become involved and stem lesions were numerous. By the 20th of August most of the leaves had turned brown and had been shed. These observations indicate that none of the varieties of bright tobacco tested are resistant to the brown-spot disease under field conditions.

Inoculation Experiments

Numerous tobacco plants in all stages of development have been inoculated and kept under greenhouse conditions. Vigorous tobacco seedlings with 4 to 6 leaves, growing in flats and pots, have been atomized with water suspensions of spores on various occasions, but in every instance little or no infection developed. Because of this fact, it seems probable, therefore, that seedlings are not very susceptible to the disease, while in the plant beds, as long as normal rapid growth is maintained. However, if the plants are stunted, they become infected quite readily at any stage of growth. For nearly all inoculation work, plants from 12 to 20 inches high in flats or pots were used.

In all inoculation experiments, the plants were placed under moist chambers immediately after they were inoculated. The moist chambers were then covered with heavy wrapping paper or burlap bags to keep out the sunlight and to keep down the extremely high temperatures which develop under glass during the midday hours. Water was sprinkled on the plants through a nozzle from a water hose once every 24 hours for 3 or 4 days to maintain a high humidity, after which time the moist chambers were removed. The incubation period under these conditions varied from 5 to 8 days. If no infection was observed within this period, the plants were either discarded or no further observations were made.

Early in the spring of 1928 a series of inoculations was begun to determine whether there existed any degree of immunity among some of the varieties of bright tobacco grown in this district. Thirty plants of each of the following varieties were used for this inoculation work: Adcock, Big Stem Orinoco, Bonanza, Cash, Crutcher, Improved Warne, Improved White Stem Orinoco, McAdoo, Narrow Leaf Orinoco, White Stem Orinoco, and Yellow Pryor. When the plants reached 14 to 16 inches in height, 9 plants of each of the 3 groups of a variety were inoculated with a water suspension of spores from cultures 20 to 30 days old. One plant of each group was kept as a check by placing a sheet of paper between it and the 9 other plants that were inoculated. After 3 days, the moist chambers were removed and placed over 3 groups of plants of another variety that had just

been inoculated with a water suspension of spores. The inoculations of this series, therefore, followed each other at intervals of 3 days. The conditions were, however, essentially the same, except for the time element.

In summarizing the results obtained by inoculating the 11 varieties of tobacco, it was observed that there were some differences in the amount of infection produced. For the want of better terms, the following adjectives are used to describe the degree of infection: "severe," "medium," and "slight."

FIG. 2. Warne tobacco plant artificially inoculated with spore suspension of *Alternaria longipes*.

Infection was considered severe when all leaves except those near the bud were involved (Fig. 2); medium when the foliage halfway up the stalk was slightly spotted; and slight when only the 3 or 4 lower leaves were spotted.

Of a total of 27 Cash plants inoculated 23 developed severe infection. The other varieties which showed severe infection were Big Stem Orinoco, Improved Warne, Yellow Pryor, White Stem Orinoco, and Narrow Leaf Orinoco. The varieties which showed medium infection were Bonanza,

Improved White Stem Orinoco, Adcock, and McAdoo. Crutcher was the only variety on which slight infection was obtained. There were some individual differences as between groups of plants of each variety, but the results shown represent the average for the variety. To judge from the results of this series of inoculations, there does not appear to be enough resistance in any one variety tested to enable a grower to escape all losses from brown spot. Figure 3 well represents the effect of Alternaria on the entire collection of plants inoculated.

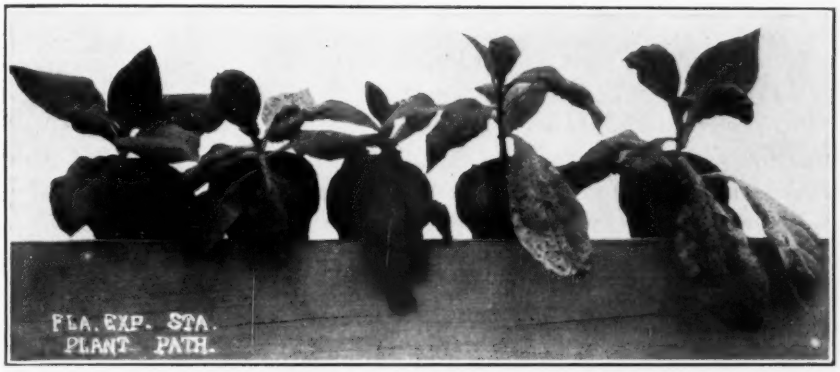

FIG. 3. Tobacco plants inoculated with *Alternaria longipes*. From left to right: Check plant (Connecticut Round Tip), Big Cuba, Big Stem Orinoco, Yellow Pryor, and Warne.

THE FUNGUS

Overwintering

One of the chief ways that this species of Alternaria appears to overwinter is on the old stalks left standing in the fields. Early in the spring of 1928 isolations were made from portions of old stalks known to have been heavily infected the previous growing season. Three different species of Alternaria were obtained from this material but only one of them proved pathogenic to tobacco. In August, 1928, 24 stalks bearing brown-spot lesions were brought from the field and stood upright in a trench near the laboratory building. On the same date several other infected stalks from the same source were cut into sections 10 to 12 inches long, wrapped in thick paper, and carried through the winter under the following conditions:

Lot No. 1 buried 6 inches deep in the soil.
" " 2 " 4 " " " " " "
" " 3 " 2 " " " " " "
" " 4 placed on the surface of the ground.
" " 5 suspended 2 feet in the air from a wire fence.

Isolations were attempted from time to time during the winter of 1928–29 from the stalks on the surface of the ground, the ones standing upright and the ones suspended aboveground. In almost every attempt, a species of Alternaria was obtained that was very similar in growth characters to the one found during the growing season.

The stalk material buried underground gave negative results when isolations were attempted. All of this material was in a partial state of decay when first examined a month after being placed in the soil. By the 11th of February, 1929, all the stalks were in an advanced state of decay and were discarded.

Spores could be seen on the lesions throughout the winter on the old stems carried over the winter in the open. This was especially true during warm, cloudy periods accompanied by rain. The temperature at such times was high enough to cause the old mycelium in the tissues of the old stalks to send out new conidiophores and produce conidia. Some of the spores produced under these conditions were irregular in size and shape. The younger spores were of the type found on the leaf lesions during the summer months, while the older ones were irregular in shape and deeply constricted at the septa. This was also observed by Elliott (2) in his studies on the genus Alternaria. These longevity studies show that sufficient viable spores are present at planting time to produce primary infection. Temperature studies of the organism in culture, as given elsewhere, show that vegetative growth and spore production occur at low temperatures.

Proof of Pathogenicity

Isolations were made from spotted leaves by W. B. Tisdale in the summer of 1925. During February and March of 1926, artificial inoculations with water suspensions of spores were made on plants in the greenhouse under moist chambers. In one flat, the lower leaves had been stunted and were somewhat yellow at the time of inoculation. After 5 to 7 days, numerous small brown spots were evident and the fungus was reisolated from the diseased areas. No further inoculations were made until December, 1926. During that month 3 Connecticut Round Tip tobacco plants, 2 feet high, were atomized with a water suspension of spores and placed under a moist chamber for 5 days. Although several leaves were punctured in one or more places with a sterile needle before they were atomized, no infection developed.

In February, 2 flats containing Connecticut Round Tip plants were inoculated with a water suspension of spores and placed under moist chambers for 3 days. At this time a number of small, incipient, water-soaked areas were observed. After 8 days numerous brown lesions had developed

on the leaves, some of which had coalesced. An Alternaria was isolated from these lesions, similar in every respect to the original organism. During February, 1927, the temperature was somewhat higher than in December, 1926, when the first inoculation was made. This may account for the negative results of the previous inoculation. Numerous other inoculations were made during the summer months and infection always developed when the plants were in a humid atmosphere. Rands (5) reported that good infection of early blight of potatoes occurred during days of high humidity and high temperature.

Effect of Temperature on the Degree of Infection

In order to determine more accurately the effect of temperature on infection, tobacco plants were inoculated at different seasons of the year and records of the temperature and humidity were kept during the incubation period. In the winter of 1927–28, a series of inoculations was begun and continued until midsummer of 1928. For this work only plants of the Cash variety were used. The relative humidity under the moist chambers in these experiments was approximately 90 per cent during the 3 or 4 days following each inoculation. This humidity was high enough to keep the foliage and glass of the moist chambers moist. The inoculations made in midwinter when the temperature was relatively low gave almost negative results, while the ones made later, when night temperatures were higher, resulted in more severe infection. The degree of infection apparently depended on the temperature for the 3- to 5-day period following the time of inoculation, thus indicating that temperature is an important factor in infection. When the average temperature was 19° C. and below, no infection was produced. As the average temperature gradually climbed to about 20.5° C., slight infection was produced on the older leaves. Medium infection was secured with mean temperatures of 23 to 25° C. and severe infection was obtained when the mean temperature was from 26.5 to 31.0° C.

There is a close relation, it appears, between the temperature at which infection developed and the mean temperatures for the months of January to August, 1928, inclusive. The mean temperature for the months of the first half of the year are as follows: January, 10° C.; February, 14° C.; March, 17° C.; April, 17° C.; May, 22° C.; June, 26° C.; July, 27° C., August, 27° C. By comparing the temperatures at which medium or severe infection was produced experimentally, with the mean temperatures for June, July, and August, one would expect abundant field infection during these months. Observations extending over a period of several years have shown that these are the months during which the disease occurs and develops in the fields. Therefore, the severity of the disease appears to be inti-

mately associated with the degree of maturity of the plant and relatively high humidity and temperature.

Method of Infection

A water suspension of spores was applied to marked portions of leaves with a loop needle and these inoculated areas were killed, clarified, and examined to determine the relation of parasite to host. After 12 to 18 hours' incubation at room temperature, these portions of the leaves were cut out and placed in Gilson's solution for 18 to 20 hours, then washed thoroughly in running water and placed in a weak solution of alcohol. This material was run through the alcohols and xylol and, after remaining in pure xylol for 3 to 4 days, the leaf tissues became transparent enough to permit light staining with eosine-lactophenol. This process permitted a view of the germinating spores and the germ tubes penetrating the epidermal cells. It was found that 1 to 4 germ tubes had been produced and these were entering the leaf through the stomata. A few germ tubes were observed entering the leaf through the basal cells of epidermal hairs and directly through the epidermis.

Morphology

Mycelium. The mycelium permeates the leaf tissue in all directions from the point of infection, passing between and through the cells. The hyphae vary in diameter on different substrata, ranging from 1 μ in the plant tissue to 4.5 μ in culture media. The older hyphae are much larger in diameter than the younger ones and have more septations. The cell walls are thicker in the older growth. In the leaf tissue the hyphae soon come near the surface and either break through the epidermis or emerge through the stomata. At this stage, the cells of the host tissue collapse, resulting in sunken brown lesions. In this condition when accompanied by high humidity, numerous conidiophores, 3 to 10, develop at one point, and produce conidia. In cultures 10 to 15 days old the entire growth becomes dark brown or olivaceous, producing an abundance of conidia.

Conidia. The young conidia are of the same color as that of the imbedded hyphae. They are ovate and slightly constricted at the septa. The cell walls become thicker and constrictions at the septa become more pronounced as the spores grow older. Conidia on the host may be much larger than those grown on culture media. From leaf spots they measure 11–13 × 30–86 μ, averaging 12 × 50 μ. The number of cross septations varies from 3 to 5, with a good percentage of spores having 1 to 2 longitudinal septations (Fig. 4, A). Spores from 20-day-old cultures, on potato-dextrose agar, measured from 6.5–13 × 25–52.6 μ, the average being about 9.6 ×

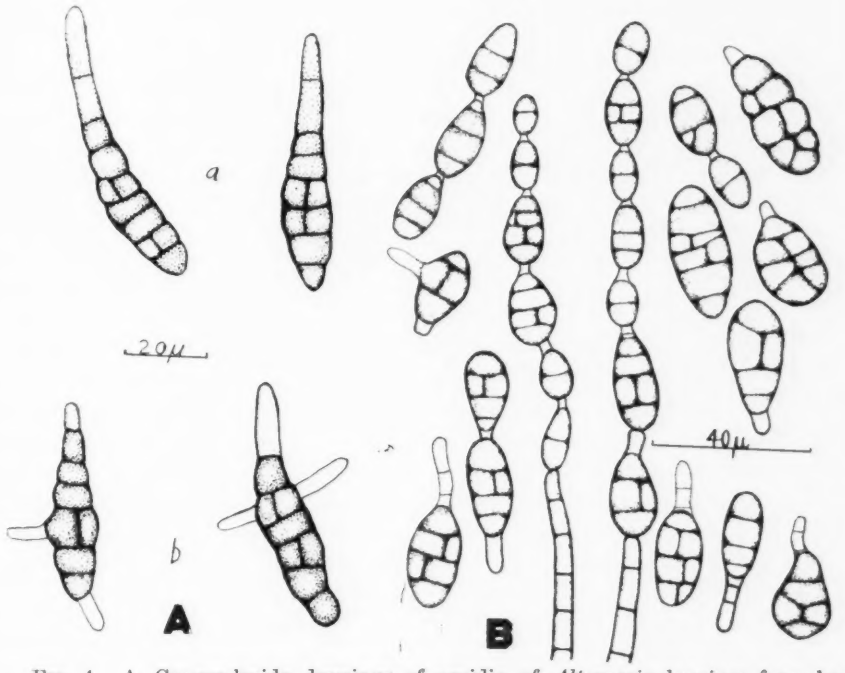

FIG. 4. A. Camera-lucida drawings of conidia of *Alternaria longipes* from host plant: a. As they appear under field conditions; b. Germinating. B. Camera-lucida drawings of conidia of *A. longipes* produced on potato-dextrose agar, showing different types and chains.

29.3 μ. The number of septations in most spores from culture media is about the same as that found on the host plant, although a few spores have only 2 or 3 cells.

Taxonomy

Ellis and Everhart (3) reported a "white speck" of tobacco from North Carolina in 1892 caused by *Macrosporium tabacinum*, n. sp. The description of this disease and causal organism as given by them is as follows:

Spots amphigenous, numerous, thin, white (rusty red or brown at first), suborbicular or irregular, 2–3 mm. in diameter, definitely limited, with a narrow darker border. Fertile hyphae effused, 35–45 × 3–4 μ, septate and torulose above. Conidia obovate, 15–25 × 10–12 μ sessile, or longer (35–45 μ) narrowed below into a distinct stipe, 8–12 μ long. The shorter conidia are mostly 3-septate and the longer ones about 5-septate, one or two of the cells with a longitudinal septum. This is the "white speck" of the North Carolina planters.

In this same reference, Ellis and Everhart described *Macrosporium longipes*, n. sp. from North Carolina as causing "brown spot" of tobacco. The description of this species is as follows:

Spots amphigenous, orbicular, rusty brown, 3–5 mm. in diameter; orbicular, zonate. The entire leaf becomes brown and then the spots are a shade lighter than the surrounding parts. Fertile hyphae effused on the spots, amphigenous, but more abundant above, slender (40–70 × 3–4 μ), septate and often constricted at the septa; erect, more or less torulose above. Conidia clavate, 40–50 × 15–20 μ, 3–7, mostly 5–6 septate, with two or more of the cells divided by a longitudinal septum, attenuated below into a distinct stipe 35–50 μ long, and often septate and torulose. This differs from *M. commune* Rabh., in its effused hyphae and smooth conidia, and from *M. tabacinum* Ell. and Ev., in its brown, concentrically zoned spots and larger stipitate conidia. Known among the planters as "brown spot."

No mention is made in literature of any inoculation experiments with either of these organisms and, as a result, their pathogenicity has been questioned (4). These two reports appeared many years ago and no recent reports of work with Alternaria or Macrosporium on tobacco have been found.

The description of the species of Alternaria found on tobacco in the Florida district is as follows:

Spots amphigenous, orbicular or slightly irregular, older ones 1.5 to 2.5 cm. in diameter, dark brown center, bordered by a band of lighter color. A narrow dark brown band or ring separates this lighter colored band from the green healthy tissue. Center of spots never turns white. Faint concentric rings are apparent in some spots (Fig. 1). Many spots are never larger than 3 to 10 mm. in diameter. When spots are numerous, coalescence takes place and a large portion of the leaf becomes brown. Fertile hyphae effused on the spots, somewhat erect, more abundant above, 35–74 × 3–4 μ. Conidia clavate to ovate, often with short beaks with 1–3 septations, 3–7 septate, one or two cells longitudinally septate, slightly constricted at the septa, 30–50 × 10–13 μ (Fig. 4, A).

In culture on potato dextrose agar the margin of growth is regular or slightly dentate: young mycelium white, turning olive green to black with age; aerial hyphae abundant, 5–7 mm. high, fluffy or tufted, bearing numerous conidiophores with catenulated spores (Fig. 4, B). Spores 3–5 septate, a few 1–2 longitudinally septate, 24–25 × 7–13 μ, slightly constricted at the septa, becoming more pronounced with age. In 30-day-old cultures, the color is dark olivaceous or almost black.

There is no exact way of determining at present the exact relation that may exist between the two species of Macrosporium reported by Ellis and Everhart (3) and the species of Alternaria found on tobacco in Florida. Some of the species of Macrosporium have been shown to produce catenulated spores, which, therefore, necessitated placing them in the Alternaria group. The only method of comparing either of Ellis and Everhart's organisms with the one considered in this paper is by considering spore measurements and the characteristics of the disease produced by each. In

comparing the spore measurements, one is likely to arrive at the wrong conclusion, since it has been shown by Elliott (2) that the dimensions and other morphological characters of spores of Alternaria in *exsiccati* may vary considerably. Then, again, the measurements of spores of the tobacco Alternaria found in Florida produced some smaller spores on culture media (potato-dextrose agar) than those found on the host plant. In considering the characters of the disease, it appears still more confusing, for some of the characters produced by each organism very closely resemble each other, while, on the other hand, there is one outstanding difference. The white spots produced by *Macrosporium tabacinum* E. & E. are not found with either *M. longipes* E. & E. or *Alternaria* sp. on Florida-grown tobacco.

In table 1, the spore and spot characters are summarized. It will be observed that spore measurements of the Alternaria herein described more closely resemble those of *Macrosporium longipes* E. & E. According to Ellis and Everhart (3), the principal differences between *M. longipes* and *M. tabacinum* are in the size of the spores and the color of the spots.

It appears from table 1 that the differences between the organism under consideration and *Macrosporium longipes* E. & E. are not sufficiently great to warrant a new species. However, because the spores are produced in chains, the new combination **Alternaria longipes** (Ell. & Ev.) is suggested.

TABLE 1.—*Summary of the primary characteristics of spores of and the diseases caused by Macrosporium longipes, M. tabacinum, and Alternaria longipes*

Organism	Spore measurements	Spore characters			Spot characters
		Cross Sept.	Longi. Sept.	Spore shape	
M. tabacinum	15–25 × 10–12 μ	3–5	1–2	Obovate stipitate	Amphigenous, red or brown later turning white.
M. longipes	40–50 × 15–20 μ	3–7	1–2	Clavate stipitate	Amphigenous, brown, zonate.
Alternaria longipes	30–50 × 10–13 μ	3–7	1–2	Clavate to ovate	Amphigenous, brown, zonate.

Physiology:

Cultural Characteristics

During the progress of this work *Alternaria longipes* has been grown on several kinds of agar culture media and on cooked stems of tobacco, sweet clover, potato, tomato, and bean pods for comparison and to determine whether a perfect stage would develop. In the course of study the following observations were made:

Potato-dextrose agar. Single germinating spores when transferred to Petri plates of this medium at room temperature produced a light olivaceous growth, measuring on an average 23 mm. in diameter by the third day. After 10 days, the increase in diameter averaged 6.25 mm. per day. Nearly all single-spore cultures in Petri dishes show a distinct zonation or concentric bands of light and dark color. After a few days dark sectors develop (Fig. 5) and, later, the entire colony becomes dark olivaceous. Aerial hyphae are abundant, somewhat in tufts or mats upon which spores are produced in abundance in cultures 5 to 7 days old.

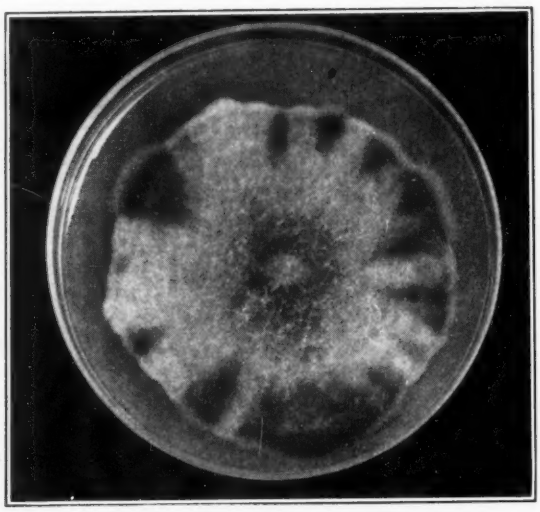

FIG. 5. Growth of *Alternaria longipes* on potato-dextrose agar at 22° after 8 days.

Oat agar. Growth on oat agar produced scant aerial hyphae, margins regular, thin and light colored, and spores relatively abundant at the end of 5 days. Single-spore plate cultures increased in diameter at the rate of 9.5 mm. per day.

Plain potato agar. The rate of growth was about the same as that on potato-dextrose agar but a much longer time was required for sporulation.

Tobacco-dextrose agar. This medium was prepared as follows: 200 gm. of tender tobacco stems were added to 500 cc. of distilled water and cooked in a double boiler for 45 minutes. To this decoction 17 gm. of bacto-agar and 20 gm. of bacto-dextrose were added and the entire volume was brought up to 1 liter, cooked, and autoclaved. The color of aerial hyphae produced on this medium was almost black. Daily increase in diameter of growth was 4 mm. Spores were very dark in color and abundant at the end of 7 days.

Sweet-clover agar. This medium was made up by using 200 gm. of sweet clover stems, 17 gm. of bacto-agar, and 20 gm. of bacto-dextrose to 1 liter of distilled water. The rate of growth and other characters produced on this medium were about the same as those observed on potato agar, except for lighter colored aerial hyphae.

Sterilized sweet-clover stems. (In Roux tubes with water and absorbent cotton on the bottom.) Growth was rapid, covering the stems in 5 to 7 days, with light olive-green growth, later turning much darker. Spores were relatively abundant at the end of 10 days. The fungus had grown to the bottom of tubes after 90 days and was producing spores on the cotton.

Sterilized bean pods. (Prepared in Roux tubes.) The growth of mycelium was vigorous, almost white at first, later turning olivaceous, and producing abundant spores at the end of 10 days.

Sterilized potato stems. (Prepared in Roux tubes.) Growth was somewhat slow, whitish at first, but, later, turning to an olive green. Spores were produced in abundance in 10 to 12 days.

Throughout all the cultural work conducted in the laboratory no evidences of a perfect stage were observed on any culture media used. The same type of spore was produced on all culture media, except that those produced on oat agar were slightly smaller, averaging $10.8 \times 25.2\ \mu$.

Effect of Temperature on the Development of the Fungus in Culture

In preliminary tests it was found that single-spore cultures made the greatest growth in diameter at pH 5.0 to 6.5, at a temperature of 24 to 27° C. Using this as a basis, all cultures were adjusted to pH 6.5 by the addition of N/1 hydrochloric acid. Only potato-dextrose agar was used for this particular work. The spores were germinated in dilution plates of potato agar kept at 23 to 24° C. and those which showed as near the same stage of germination as could be obtained were used to inoculate sterile Petri plates. All cultures for temperature studies were run for 8 consecutive days. The cultures from 25 to 35° C. were kept in a Freas oven with the temperature varying not over 1 or 2°. Cultures kept at 29–31, 32–33, and 34–35° C. were placed in a moist chamber to prevent rapid desiccation of the culture media. Cultures exposed at from 10 to 25° C. were kept in various places in a refrigerator. Under this condition, fluctuations in temperature were greater than with cultures placed in the Freas oven at higher temperatures.

Table 2 shows that the fungus grows in culture media over a fairly wide range of temperature. The optimum temperature for growth, based upon rate of increase in diameter of colony, lies between 25 and 29° C. The decrease in rate of growth is very rapid from 29 to 35° C. where no growth occurred. After germinated spores had been kept at 35° C. for 24 hours

they failed to make any growth in 3 days when transferred to another incubator kept at 25° C. Mature spores in dilution plates containing potato-dextrose agar germinated within 5 hours at 35° C., but when the germ tubes attained a length about equal to the diameter of the spore no additional growth occurred. From the optimal temperature down to 10 degrees, the decline in growth was more gradual. Because of the lack of facilities, the minimal temperature could not be determined but, judging from the growth made at 9–11°, it is apparently not much below 9° C. Spores of the usual size and shape were produced in abundance near the optimal temperature. Sporulation also occurred in cultures kept at higher temperatures, but the spores were smaller and more irregular in shape. Spore formation was retarded toward the lower end of the temperature range.

TABLE 2.—*Diameter of single spore cultures of Alternaria longipes on potato-dextrose agar exposed for 8 days at temperatures indicated*

Temperature °C.	No. plates	Average diameter of growth in mm.
9–11	20	8.3
11–13	22	13.5
12–14	24	18.7
14–16	24	25.3
17–19	25	33.6
18–21	20	41.2
21–23	24	49.2
25–26	35	53.2
26–28	30	58.0
29–31	35	25.0
32–33	45	9.8
34–35	45	0.0

The range of temperature for spore germination is almost identical with that for vegetative growth and sporulation. About 53.2 per cent of the spores germinated at the end of 36 hours at 9–11° C. Only 1 or 2 germ tubes developed at this temperature and 24 to 28 hours were required for them to attain a length equal to the diameter of the spore. At 11–14° C., the time for germination was 20–22 hours with 59.6 per cent germination. From 16–20° C., the time for germination was reduced to 14 to 16 hours

with 73.4 per cent germination. At temperatures ranging from 21–25° C., 78.3 per cent germination was secured within 10 hours. The most favorable temperature for germination was between 26–29° C., where 93.4 per cent of the spores germinated within 8 hours. From 30–35° C., the time for germination was reduced to 5 hours with practically all the cells of the spores sending out germ tubes. The percentage of germination at these higher temperatures was not appreciably different from the figures obtained at 26–29° C.

Effect of Hydrogen-ion Concentration of Culture Media upon the Rate of Growth of Alternaria longipes

Potato-dextrose agar was used in the experiments to determine the effects of hydrogen-ion concentration upon the rate of growth of *Alternaria*

TABLE 3.—*Growth of single-spore cultures of Alternaria longipes in 8 days at 26° C. on potato-dextrose agar of pH concentration indicated*

pH value of culture media	Growth in diameter—mm.	Growth characteristics
1.50	No growth	
2.00	Very little growth	White, warty growth. No spores produced.
2.50	3.2	White, warty growth. No aerial mycelium produced. Becomes sooty white with age. No spores.
3.00	7.5	Sooty white, very little aerial mycelium. Spores few and irregular in shape.
3.50	13.6	Growth of grayish color, aerial mycelium abundant. Spores more abundant than at pH. 3.00.
4.00	20.1	Growth almost black in color, aerial mycelium abundant. Spores abundant. Margins regular.
4.50	45.0	do
5.00	50.4	do
5.50	58.5	do
6.00	55.2	do
6.50	43.7	do
7.00	48.5	Color typically olivaceous. Margins more or less irregular. Aerial hyphae relatively abundant.
7.50	54.0	do
8.00	45.8	Growth much lighter in color. Margins almost regular. Spores abundant, regular in shape and color.
8.50	42.3	do
9.00	31.6	do

longipes. The cultures were run in quadruplicate at each pH reaction and the experiment was repeated. The measurements shown in table 3 are the average for the two series. Two liters of potato-dextrose agar containing 2.2 per cent bacto-agar were prepared for each series. The medium was divided into the several lots to be adjusted to various pH values, ranging from pH 1.5 to pH 9.0 by the addition of N/1 hydrochloric acid and N/1 sodium hydroxide. Determinations were made by the use of a quinhydrone potentiometer. Petri plates were inoculated in the same manner as that described in temperature studies on culture media. The plates were then exposed to a constant temperature of 26° C. for 8 days. At the end of this period, measurements and observations were made and are summarized in table 3.

From these results it will be observed that, so far as diameter of growth is concerned, the most favorable reactions were obtained with pH values between 4.50 and 8.00.

CONTROL MEASURES

In discussing control measures for brown spot of tobacco, it will be well to keep in mind that work done towards prevention is far more important than trying to check the disease in a field after the plants once show signs of infection. Since spraying or dusting the plants would add considerable expense in both labor and materials to a crop that is more or less uncertain in its returns and since spray or dust materials are objectionable on the cured leaf, it seems more advisable to resort to sanitary measures and improvement of cultural methods. When harvesting is complete, the stalks should be cut with a stalk cutter and turned under to bring about complete decay as quickly as possible. If these operations are inconvenient just after harvesting the top leaves, the stalks may be cut and turned under later in the season.

Crop rotation might be used advantageously in combating the disease, because it will not only reduce to a minimum the chances for primary infection of brown spot but will also reduce the chances for infection by other parasites. Since brown spot has been observed to be serious on plants affected with root knot, it is advisable to adopt a rotation system which will reduce root-knot infection to a minimum. Cultural methods should be so regulated as to keep the plants in a vigorous state of growth until they are mature; then, the leaves should be primed as they become ripe or mature. Most of the natural infection has been observed to occur on overripe leaves or on leaves that have been prematurely ripened because of root knot or insufficient water supply.

SUMMARY

1. A leaf-spot disease of bright or flue-cured tobacco, commonly known as brown spot, has been prevalent for several years in the Florida-Georgia tobacco district.

2. A similar, if not the same, disease was reported by Ellis and Everhart in 1892 from North Carolina.

3. In comparing reports of losses caused by various tobacco diseases in this district, it will be noted that, while the brown-spot disease is not of major importance, yet, during certain seasons when ideal conditions prevail for the activity of the fungus, considerable reduction in both price and yield is sustained by the growers.

4. The disease appears first on the lower leaves as small spots which rapidly enlarge and become brown. If the spots are numerous, coalescence takes place and the entire leaf may turn brown. Under very favorable conditions the disease spreads rapidly to the upper leaves and causes a complete loss of the crop.

5. So far as has been observed in fields and also from inoculation experiments, tobacco is the only plant susceptible to infection.

6. There appears to be very little difference in the susceptibility of the different varieties of bright tobacco tested.

7. The organism hibernates on the stalks left standing in the fields or lying on the surface of the ground.

8. Proof of the pathogenicity of the organism has been fully established by numerous inoculation experiments.

9. The mode of infection is by direct penetration of the epidermal cells and through the stomata, wounding being unnecessary for infection.

10. The organism herein reported produces catenulate spores, comparable in size to those of *Macrosporium longipes* E. & E., and the character of the spots is almost identical. Because of catenulate spore formation, however, the name **Alternaria longipes** (E. & E.), new combination, is proposed.

11. The minimal temperature for infection is about 20 to 22° C. Medium infection was produced when the average temperature was from 23 to 25° C. Severe infection was produced when the average temperature was 26.5° C. and above. The maximal temperature for infection was not determined.

12. The optimal temperature for the development of the fungus on culture media was between 25 and 28° C. The maximal temperature is near 34–35° C. The minimal temperature for the development of the fungus in culture was not determined.

13. The optimal temperatures for most severe infection and for growth of the fungus in culture are approximately the same.

14. Maximal amount of growth on potato-dextrose agar was secured at pH 5.5. There was a slight decrease in growth with pH values 6.0 and 6.5 but an increase again with pH 7.0 and 7.5.

15. The stalks should be cut and turned under immediately after the crop is harvested to minimize the source of inoculum the following year. Good fertilization, together with crop rotation and frequent cultivation, is advisable. The leaves should be primed when they reach the proper stage of maturity.

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FURTHER STUDIES ON REACTION OF CORN TO SMUT AND EFFECT OF SMUT ON YIELD[1]

F. R. Immer and J. J. Christensen

INTRODUCTION

Breeding for disease resistance seems to be the only promising method for controlling corn smut, *Ustilago zeae* (Beckm.) Ung. The chlamydospores are produced in enormous numbers and can live in the soil for several years; therefore, inoculum will always be present in abundance in the corn-growing area. Part of the life cycle of the organism can be spent as a saprophyte in the soil and part as a parasite on corn plants. Adequate control by cultural practices is, therefore, practically impossible.

It has been shown that inbred lines of corn, quite resistant to smut, can be produced rather easily. The rapidity with which these selfed lines become homozygous for reaction to smut suggests that a relatively small number of genetic factors are involved in determining smut reaction. Breeding for resistance to smut by selection in self-fertilized lines seems to be the most promising method for controlling the disease.

REVIEW OF LITERATURE

Extensive tests have demonstrated that inbred lines of corn which differ markedly in resistance to smut (3, 5, 7, 8, 9) can be produced. The reaction of these inbred lines to smut infection has been found to be quite constant from year to year. Inbred lines can be produced with every degree of resistance or susceptibility, when grown in an epiphytotic of the disease. Kyle (13) has shown that a tight husk covering favors resistance to ear infection.

Several controlled experiments have been conducted to determine the damage caused by smut galls on parts of corn plants other than the ears. Analyses were made on the basis of barrenness induced by the fungus and reduction in yield of shelled corn caused by smut galls on the plants. Garber and Hoover (4) found that smut infections increased the percentage of barren plants, but there was no significant decrease in yield due to smut infection apart from the greater percentage of barren stalks. Immer and Christensen (10) and Jorgenson (12), working in Minnesota and Ohio, respectively, used essentially the same methods and found that smut galls on the plants of inbred lines reduced yields very materially. Jorgenson found essentially the same condition in F_1 crosses.

[1] Contribution from the Division of Agronomy and Plant Genetics and the Division of Plant Pathology and Botany, University of Minnesota, St. Paul, Minnesota.

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It has been demonstrated in a number of instances that smut collections from different localities differed in their ability to cause infections when the plants were inoculated with a hypodermic syringe or when inoculum was dropped in the apical heads of young corn plants (1, 2, 6, 17). Smut collections made in different States or in different parts of the same State or locality have sometimes been found more virulent than local smut collections under these conditions. No extensive tests have been reported previously giving the reaction of corn strains to local and nonlocal collections under natural field conditions. In view of the demonstration of heterothallism and frequent mutation in *Ustilago zeae*, the constancy of reaction of strains of corn to natural infection by local and nonlocal collections of the organism the same year or in successive years is of extreme importance. Mutations have been shown to occur in monosporidial lines of the smut organism in surprisingly large numbers (1, 15, 16). These mutants can then hybridize and new combinations of characters can be produced (16), since *U. zeae* is heterothallic (14).

Jones (11) found resistance to smut infection to be dominant in the F_1 generation of crosses between selfed lines that differed in smut reaction. Hayes *et al.* (7) found F_1 crosses between resistant strains slightly more resistant than either parent, while F_1 crosses between resistant and susceptible strains were intermediate in reaction. Immer and Christensen (9) corroborated the conclusion reached in the earlier Minnesota studies that dominance of resistance or susceptibility was lacking in F_1 crosses between inbred lines differing in smut reaction. In later studies, Immer (8) again reported F_1 crosses to be intermediate in reaction, although several cases of apparent dominance of susceptibility were found. A slight tendency to approach the more susceptible parent was found in back-crosses and in F_3 populations.

The purpose of the present paper is to present further data on the determination of losses due to smut in corn, to compare the reaction of inbred lines to natural infection in epiphytotics from local and nonlocal smut collections, and to determine further the mode of inheritance of resistance or susceptibility to smut in crosses between inbred lines.

MATERIALS AND METHODS

The selfed lines and crosses of corn used in all parts of this study, except those dealing with losses due to smut infections, were grown in two systematically distributed plots of 30 to 40 plants each. A smut epiphytotic was induced in these plots and notes were taken on size and location of smut infections at two or three times during the growing season, as described previously (8, 9).

The study of losses due to smut infections in selfed lines was conducted as described previously (10), i.e., the yield of oven-dry, shelled corn from two plants growing one foot apart was compared, one plant bearing a single smut gall and the other plant of the pair being smut-free. These data were collected in selfed lines, inbred for five or more generations. Notes were taken on location of galls on the plants as well as size of galls.

The study of smut-induced losses in F_1 crosses of corn was made in the regular yield trials of these crosses. Two plants were selected from a hill of three, one plant being infected with a single gall and the other being free from smut galls. Notes were taken and the results analyzed in the manner described for selfed lines.

In 1928 and 1929 all strains of corn tested in the "smut plots" were grown in two replicated single-row plots in each of two widely separated fields. In field E a smut epiphytotic was created, using smut collected at University Farm, St. Paul, Minnesota, only. Part of the field had been used as the corn smut plot for each of 2 years prior to 1929 and the other part had been so used for the 8 years preceding 1929. Conditions in this plot were, therefore, very favorable for smut infection. In another field, X–28, separated from field E by a distance of more than 1 mile and by certain natural barriers to the spread of smut spores from one field to the other, the same strains were grown in an artificially induced epiphytotic of smut collected from numerous sources in the northern Mississippi Valley as well as at University Farm. Many of the forms of smut present in field E probably were present in X–28, as well. The purpose of this phase of the study was to compare the reaction of selfed lines and crosses of corn to natural infection by local collections of the smut organisms with their reaction to a combination of both local and wide smut collections.

EXPERIMENTAL RESULTS

Losses due to smut: The losses induced by smut galls located on the corn stalks is of interest to plant pathologists and plant breeders, alike. The results of the experiments to determine the losses due to single smut galls of different sizes and on different parts of the plants, apart from the ears, covering a period of 3 years in the case of selfed lines and 2 years for F_1 crosses, are given in table 1.

Losses due to smut galls appear to be directly correlated with the size of the galls. The data on damage due to large and small galls both above and below the ears indicate that single large galls (4 in. or more in diameter) reduce the yield of shelled corn on the infected plants approximately four times as much as small galls (less than 2 in. in diameter).

There seems little difference in damage induced by smut galls at the base or first shoot as compared with the losses from smut at higher shoots

TABLE 1.—*Loss in shelled corn due to single smut galls on plants of selfed lines or F_1 crosses. Data on selfed lines collected 1927–29 and F_1 crosses 1928–29*

Location of galls	Size[a]	Selfed Lines			F_1 Crosses		
		Number of comparisons	Per cent loss	Odds that loss is significant	Number of comparisons	Per cent loss	Odds that loss is significant
Base and first shoot	L	14	55	1499:1	3	26	14:1
" " " "	M	47	21	189:1	8	26	13:1
" " " "	S	35	12	42:1	6	15	6:1
Second and higher shoots	L	25	57	>10000:1	13	67	1833:1
" " " "	M	31	18	38:1	16	21	30:1
" " " "	S	23	18	44:1	9	5	3:1
Neck	L	21	99	>10000:1	21	58	>10000:1
"	M	27	45	1999:1	57	33	>10000:1
"	S	24	−4	2:1	49	15	294:1
"	All	72	45	>10000:1	127	31	>10000:1
Total infection below ear	L	43	61	>10000:1	17	54	>10000:1
" " " "	M	80	22	>10000:1	25	23	108:1
" " " "	S	61	15	1193:1	15	9	12:1
" " " "	All	184	29	>10000:1	57	31	>10000:1
" " above "	L	38	95	>10000:1	22	59	>10000:1
" " " "	M	40	41	>10000:1	58	33	>10000:1
" " " "	S	37	1	1:1	53	15	591:1
" " " "	All	115	47	>10000:1	133	31	>10000:1

[a] L = Large galls; *i.e.*, 4 in. in one or more diameters.
M = Medium galls; *i.e.*, larger than "small" and smaller than "large" galls.
S = Small galls; *i.e.*, less than 2 in. in any diameter.
All = All galls at a given location.

or ear buds on the plants of selfed lines. Jorgenson (12) found this same condition in Ohio. The data from F_1 crosses were too meager to draw any conclusions on this point.

Large or medium smut galls above the ears of selfed lines did greater damage than galls of similar size below the ears. Large galls on the necks of plants from inbred lines reduced yields by 99 per cent. Such large galls resulted in barren stalks in almost every instance. In F_1 crosses infections above the ears also did slightly greater damage than infections below the ears. The difference was not so great, however, as in selfed lines. Jorgenson (12) also found that galls above the ear in F_1 crosses caused greater losses than galls below the ears.

There seems to have been little difference in the reduction in yield in selfed lines and F_1 crosses due to smut galls below the ears. Large and medium galls above the ears were more destructive in selfed lines than in F_1 crosses. Damage is dependent, to a large extent, on the size of the galls. For this reason comparisons of losses due to smut galls on different parts of the plant must be made on the basis of galls of definite size. For example, in the data given in table 1, 30 per cent of the galls below the ear in F_1 crosses are seen to be large galls, while only 17 per cent of the galls above the ear were large. Obviously, the average infection of all galls at these two locations does not give a critical comparison of the losses because a greater percentage of large galls were involved in the first instance and large galls result in greater losses than do small ones. Therefore, analyses of damage caused by smut must be made on the basis of both size and location of infections.

An indication had been found previously (8) that very susceptible selfed lines tended to have a greater number of smut galls than resistant strains. A study was made of the inbred lines grown from 1924 to 1929, this time dealing with the size of galls on resistant and susceptible strains. Only plants with a single smut gall were considered in this study. In the selfed lines with less than 15 per cent of the plants smutted, 11 per cent of the infected plants had large galls, 43 per cent medium, and 46 per cent small galls. In the selfed lines with more than 50 per cent of the plants smutted, 25 per cent of the galls on the infected plants were large, 49 per cent medium, and 26 per cent small. It is evident, therefore, that susceptible strains of corn not only tend to have more smut galls per plant but these galls tend to be larger on infected plants in susceptible than in resistant strains.

Considering that the losses due to smut are directly associated with size of gall, the notes on percentage of plants infected by smut is a very conservative measure of the relative resistance of different strains. The difference in damage due to smut in resistant as compared with susceptible

strains is considerably greater than would be indicated by the note on percentage of plants infected.

From the data given in table 1 it is possible to develop a more accurate technique than has been heretofore available for estimating the losses caused by smut in making disease surveys. Losses from ear smut can be readily determined by estimating the percentage of ears destroyed. To this must be added the damage due to smut galls on other parts of the plants. On the basis of the data available this can be done by estimating the losses in shelled corn traceable to large galls on the infected plants at 50 per cent, by medium galls at 25 per cent, and small galls at 10 per cent. The actual losses (Table 1), are but slightly greater. If it is not convenient to classify the galls on the basis of size in making the disease survey it seems safe to estimate the losses, due to all galls on the stalks of infected plants, at 30 per cent. Estimates in which size of gall is considered will, however, be more accurate.

REACTION OF SELFED LINES TO LOCAL AND NONLOCAL COLLECTIONS OF SMUT

Since corn smut causes great losses in yield of grain it is not only of great interest to determine the constancy of reaction of resistant selfed lines to smut but to determine if such selfed lines will react in the same manner to local and nonlocal collections of the smut organism. In table 2 is given the smut reaction of 15 selfed lines to local smut collection over a period of 6 years. Each of these strains has been inbred for at least 8

TABLE 2.—*Smut reaction of selfed lines grown in a smut epiphytotic for 5 or 6 years*

1929 Culture No.	Variety	Years selfed	Percentage of smut						
			1924	1925	1926	1927	1928	1929	Ave.
S–1	Rustler	10	84	100	89	100	76		90 ± 4
–4	Minn. No. 13	10	74	97	52	46	13	33	53 ± 4
–6	Longfellow	8	52	77	69	79	63		68 ± 5
–7	"	10	27	27	17	46	18	21	26 ± 3
–8	"	10	15	9	10	13	2	6	9 ± 2
–9	King Philip	8		100	78	100	76	99	91 ± 4
–10	Rustler	9	71	82	80	100	56		78 ± 4
–11	"	11	12	20	16	23	13	22	18 ± 3
–12	"	9	13	10	17	22	23	21	18 ± 3
–13	"	10	10	4	10	5	4	0	6 ± 2
–14	"	9		9	8	0	3	2	4 ± 2
–15	"	10		9	2	7	3	0	4 ± 2
–20	Minn. No. 13	8	2	20	16	16	7	0	10 ± 2
–21	"	8		0	3	0	0	0	1 ± 2
–31	N. W. Dent	8	4	9	5	9	1	4	5 ± 2

generations. The uniformity of reaction to local smut collections under field conditions is demonstrated clearly.

Selfed lines, differing greatly in resistance to smut, can be obtained in any of the varieties of corn used in breeding work at University Farm. These selfed lines usually breed true for a particular manner of reaction to smut, particularly after they have been selfed for a number of generations and approach homozygosity.

In 1928, 358 and in 1929, 281 selfed lines of corn were grown in two systematically replicated plots in each of two fields at University Farm. In field E, an artificial epiphytotic was created, using smut collected at University Farm, only. In field X–28 an artificial epiphytotic was created in which numerous smut collections from the northern Mississippi Valley,[3] including collections from field E, were used. Many of the smut forms present in field E probably were present in field X–28. The two fields were separated by a distance of more than 1 mile and by certain natural barriers.

A comparison was made of the reactions of these selfed lines to local collections of smut and to the local and nonlocal smut collections by means of correlation studies. If the correlation between the reaction in the two replicates on a single field is of essentially the same magnitude as the correlation between single replicates of the two different fields, it seems safe to conclude that the selfed lines reacted in essentially the same manner to the different smut collections. If, however, the correlation between smut reaction in the two fields was much less than between replicates on the same field, either the different physiologic forms of smut encountered affected the reaction of the selfed lines in a differential manner or cultural conditions in the two fields may have been sufficiently different to cause the seemingly differential response. The results of the study are given in table 3.

TABLE 3.—*Correlation between smut reaction of selfed lines grown in two replicates in two different fields; field E being inoculated with smut from University Farm and field X–28 with smut collected from many sources in the northern Mississippi Valley, including University Farm*

Nature of correlation	Correlation coefficient	
	1928	1929
First and second replicate in field E	+.60 ± .02	+.65 ± .02
" " " " " " X–28	+.60 ± .02	+.65 ± .02
First replicate in E with first replicate in X–28	+.36 ± .03	+.63 ± .02
Second replicate in E with second replicate in X–28	+.39 ± .03	+.68 ± .02
Average of both replicates in E with both replicates in X–28	+.47 ± .03	+.75 ± .02

[3] The writers wish to express their thanks to C. Anderson, R. Bulger, W. Butler, G. Curran, G. Mayouse, L. Person, A. Thiel, H. Thornberry, J. Wallace, and others for their kindness in supplying collections of the smut.

In 1928 the soil was very dry at planting time. Field E was planted first and the corn germinated fairly well. Field X 28 was planted 2 days later. As a consequence of this delay and the fact that field X–28 was not in so good a state of tilth at planting time, most of the seed in X–28 did not germinate until rain came, almost 2 weeks later. The delay in germination of the seed in field X–28 may account for the lower correlation between individual series of the two fields as compared with the correlation between series of the same field. It is known that the reaction of corn to smut is affected by the stage of development of the corn plants. The possibility of different physiologic forms of smut affecting the reaction of these corn strains and causing part or all of this difference is not, however, overlooked.

In 1929 soil conditions at planting time were more favorable. Both fields were planted within a period of 2 days and developed in a very comparable manner. The results obtained in 1929 appear to be more truly representative of the similarity or difference in response of these corn strains to nonlocal collections of smut. The fact that the correlation between individual replicates of the two different fields is essentially the same as the correlation between the two replicates on the same field indicates clearly that these selfed lines of corn reacted in the same manner to the two smut collections in 1929. In examining the correlation tables, showing the relationship between reaction in fields E and X–28 for both 1928 and 1929, it was noted that the reactions of a few lines, to the different smut collections, were somewhat at variance with those of the bulk of the selfed lines, as indicated by their rather wide departure from the regression line. The regression line showed the general trend between reaction of these strains in the two fields in each of the 2 years. In the case of each strain departing widely from the general trend between the two fields and grown both years, it was found that this wide departure from the regression line held for only 1 year, indicating that such departures from the average reaction were due to chance fluctuation and were not inherited from year to year. This furnishes additional evidence that the corn strains grown in 1929 reacted in essentially the same manner to smut collections made at University Farm and to collections of smut from a wider source when tested for reaction to natural infection in the field and subject to an epiphytotic of the disease.

It should, however, be emphasized that these conclusions are based on the general reaction of a large number of selfed lines to the two different smut collections. As only 30 to 40 plants were grown per replicate it was not possible to determine whether some few lines did react in a differential manner. The evidence available from this study indicated that, if there was a differential response by a few selfed lines to the two different smut

collections, this differential reaction was not very great. It should be remembered, however, that these conclusions are based on 2 years' results and that further tests are in progress. If similar results are obtained from other studies, it will greatly facilitate the production of smut-resistant corn.

REACTION OF F_1 CROSSES

Since the immediate solution of the corn-breeding problems probably will be the production and distribution of desirable hybrids between selfed lines, the reaction of F_1 crosses, as compared with that of the parental inbred lines, is of considerable importance. Eighteen such crosses between inbred lines of known reaction to smut were made in 1928. These crosses were grown in 1929 and their reaction compared with that of the parent cultures. Selfed lines with an average of 0 to 15 per cent of smutted plants were designated as low-smut lines, those with 15 to 50 per cent of smutted plants were called medium-smut lines and lines with more than 50 per cent of smutted plants were designated high-smut lines. The results are given in table 4.

In the 8 crosses between inbred lines differing in smut reaction, i.e., crosses of high x low or medium x low smut lines, 4 crosses were more resistant and 4 more susceptible than the average of the parents. This difference, however, was not appreciable, considering the magnitude of the probable errors. In the 10 F_1 crosses between low-smut lines, 9 were slightly more susceptible and 1 slightly more resistant than the average of the parent inbred lines. The greater susceptibility of the F_1 as compared with the average of the parents in low x low-smut crosses may be due to different genetic factors determining smut resistance in the different parents or to the fact that the more vigorous plants in the F_1 crosses may be slightly more susceptible than the smaller plants in the inbred lines. This latter possibility was tested by calculating the correlation between smut reaction and height of plants in 300 F_3 lines from a composite cross of 8 inbred lines. A significant correlation was not found.

Combining this group of F_1 crosses with those reported previously from Minnesota, 34 F_1 crosses between inbred lines of known smut reaction have been tested. The general conclusion to be drawn is that the F_1 tends to be intermediate in reaction.

COMPOSITE CROSS OF EIGHT SELFED LINES

In 1925, a composite cross was made of 8 supposedly low-smut lines. One of these proved to be medium in reaction in subsequent tests but the other 7 have remained quite resistant. The recombination was made by pollinating each line in turn with a mixture of pollen from the other 7. The reaction of the F_1 has been reported previously (8). A number of F_1

TABLE 4.—*Reaction of parent selfed lines and F_1 crosses of corn to smut infection*

1928 Cross	Variety	Per cent smut in parent selfed lines								Type of cross	Per cent smut in in F_1
		First parent				Second parent					
		1926	1927	1928	1929	1926	1927	1928	1929		1929
1600 × 300		64	100	92	90	12	16	6	13	High × Low	62 ± 7
1600 × 1700		64	100	92	90	.....	21	4	4	" × "	35 ± 11
600 × 1500		76	83	38	.....	30	14	8	8	" × "	52 ± 11
1200 × 100		71	75	32	95	16	14	0	2	" × "	27 ± 9
1100 × S–3		70	86	39	71	0	.....	6	.....	" × "	24 ± 9
400 × S–3		49	.....	27	.....	0	.....	6	.....	Med. × "	9 ± 4
S– 3 × S–15	Rustler	0	.....	6	.....	8	0	3	2	Low × "	18 ± 7
S– 3 × S–16	"	0	.....	6	.....	2	7	3	0	" × "	6 ± 4
S–14 × S–15	"	10	5	4	0	8	0	3	2	" × "	16 ± 7
S–14 × S–16	"	10	5	4	0	2	7	3	0	" × "	6 ± 4
S–15 × S–16	"	8	0	3	2	2	7	3	0	" × "	18 ± 7
S–C × S–8	Longfellow	13	.....	39	38	10	13	2	6	Med. × "	21 ± 9
S–23 × S–28	Minn. No. 13 ...	3	0	0	0	27	0	35	2	Low × "	18 ± 7
S–25 × S–28	" ...	0	0	0	2	27	0	35	2	" × "	24 ± 9
S–B × S–A	N. W. Dent	.....	19	10	20	5	9	1	4	Med. × "	37 ± 11
S–F × S–G	Silver King	9	4	14	19	2	11	12	9	Low × "	12 ± 7
S–F × S–H	" "	9	4	14	19	6	10	4	.....	" × "	19 ± 7
S–G × S–H	" "	2	11	12	9	6	10	4	.....	" × "	11 ± 7

TABLE 5.—*Smut reaction of eight parent lines and F_1, F_2, F_3, and F_4 generations after a combination of these selfed lines*

	Year	Frequency distribution of lines with regard to per cent smut						No. lines
		0.0–10.0	10.1–20.0	20.1–30.0	30.1–40.0	40.1–50.0	50.1–60	
Parent lines	1926	4	3	....	1	....	....	8
F_1 "	"	3	3	1	1	....	....	8
Parent lines	1927	2[a]	2	2	....	....	1	7
F_2 "	"	1	6	12	9	3	3	34
Parent lines	1928	5	2	1	....	....	....	8
F_3 "	"	109	112	40	23	8	7	299
Parent lines	1929	4[b]	....	2	....	....	....	6
Sel. F_4 "	"	52	45	26	10[b]	2	1	136

[a] No test of one parent line in 1927. Should have fallen in this class on basis of past performance.

[b] No test of two parent lines in 1929. Should have fallen in these two classes on basis of past performance.

ears were selfed and 34 F_2 lines were grown. A large number of F_2 plants were then selected at random and selfed in order to provide a large, random F_3 population. The most desirable F_3 lines, as judged from notes on plant and ear characters as well as smut resistance, were carried into F_4 by selfing. The F_4 lines, were, therefore, selected material. The smut reaction of the F_1, F_2, F_3, and selected F_4 lines was determined in a smut epiphytotic in relation to the reaction of the parent cultures. The results are given in table 5.

The F_1 recombination lines reacted essentially as would be expected on the basis of previous studies with F_1 crosses, i.e., like the average of the parents. The F_2 lines were somewhat more susceptible, in general, than the F_1. In 19 of the 34 F_2 lines less than 30 per cent of the plants were smutted, and in the other 15 lines more than 30 per cent were infected, while only 1 of the 8 parent lines had more than 30 per cent smutted plants.

The reaction of the 299 F_3 lines, grown from selfed seed of a large number of F_2 plants selected at random, is of greatest interest. Seventy-four per cent of the F_3 lines had less than 20 per cent, 87 per cent had less than 30 per cent smutted plants, and 95 per cent less than 40 per cent. It seems, therefore, that these F_3 lines reacted very nearly the same as the average of the parents. One parent was known to be a medium-smut line. The probable error of the reaction of a "30 per cent smut" line was about 10 per cent. Chance fluctuation could, therefore, account for the reaction of some of the apparently susceptible F_3 lines. In the main the same genetic factors seem to have controlled resistance to smut in those 8 parent selfed lines. The most susceptible F_3 lines were discarded in 1928 and only the more resistant lines continued in 1929. Only 3, or 2 per cent, of the F_4 lines had more smut than any of the parent inbred lines. The smut epiphytotic of 1929 was somewhat more severe than that of 1928 but the effect of the elimination of the most susceptible F_3 lines is clearly indicated. Apparently, lines can be produced very easily from recombinations of low-smut strains which are as smut resistant as the parent cultures.

SUMMARY

1. A study was made to determine the losses caused by smut infection in F_1 crosses and in selfed lines of corn inbred five or more generations.

2. Size and location of smut galls on the plant were important. The larger the galls on the stalks, the greater was the reduction in yield of shelled corn. Large or medium-size smut galls on the stalk above the ears did greater damage than galls of similar size below the ears.

3. Susceptible lines of corn tend to have a greater number of smut galls per plant and larger galls than resistant lines.

4. From the available data it was estimated that the reduction in yield in shelled corn resulting from large, medium, and small galls on stalks was 50 per cent, 25 per cent, and 10 per cent, respectively.

5. Selfed lines that differ greatly in resistance to smut can be obtained in all varieties of corn used in breeding work at University Farm.

6. Fifteen lines of corn inbred for at least 8 years reacted in a very similar manner for each of 6 different years when grown in an epiphytotic of smut at the University Farm.

7. In 1928 and 1929 the extent of infection of numerous lines grown in the field in which smut collections from University Farm only were used was correlated with the infection obtained in a different field where smut collections were from a wider source. The calculated correlation coefficients were $.47 \pm .03$ and $.75 \pm .02$, respectively.

8. The smut infection of 34 F_1 crosses was compared with that of the parents. Crosses between two resistant lines were fairly resistant, although they were more severely infected than the parents.

9. A composite cross was made of seven low-smut lines and of one medium-smut line. From a study of 299 F_2 lines selected at random 87 per cent were no more susceptible than the most susceptible parent. The results indicate that composite crosses will undoubtedly yield smut-resistant lines in a large percentage of cases.

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THE RELATION BETWEEN INSECT AND VIRUS AS SHOWN IN POTATO LEAF ROLL, AND A CLASSIFICATION OF VIROSES BASED ON THIS RELATION

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Soon after Oortwijn Botjes, Schultz, *et al.* had realized the importance of aphids in spreading potato leaf roll and mosaic, they began to investigate the part played by other insects in the spread of these diseases. These investigations, however, led to no positive results (16, p. 55; 19, p. 329; 21, p. 50).

In 1922 Murphy (13) believed that he had succeeded in proving that leaf hoppers, capsid bugs, and probably the potato flea beetle are able to transmit leaf roll and from field observations deduced that these insects play an even more important part than aphids, the latter being less numerous. His research method, however, was such that the results cannot be considered as altogether trustworthy. He used for each of the abovementioned species only one experimental plant and, during their development, the plants were not inspected for the posibility of other infections. With later experiments he and others obtained widely different results. Moreover, Elze (5) proved by experiment and field observation that the significance of aphids, especially of *Myzus persicae* Sulz., should not be sought so much in their large increase on the potato during the summer as in the abundance of the first alate generations which in early summer pass from other plants on to the potato. These winged generations are, however, rarely so numerous as to be conspicuous with field observations.

In 1927 Smith (25) published his first investigations on the significance of various sucking insects in the spreading of mosaic. He made use of some species of aphids, white fly, leaf hoppers, and capsid bugs, and found that in the given order the transmitting power decreased. He tried to explain this from the regularity with which these insects tap the phloem with their stylets. On this subject he published a separate article in 1926 (24). In this paper he starts with the supposition that all these insects transmit the virus purely mechanically but does not mention the grounds on which this supposition is based. Observations on virus diseases of various other plants do not lend support to this theory.

In later researches on the spreading of leaf roll and mosaic by the principal insects mentioned above and also by the potato flea beetle Smith (26, 27) succeeded in getting positive results only with *Myzus persicae*. His conclusion that other potato-virus workers apparently succeed more easily in transmitting these diseases with various insects would not appear ex-

actly correct. In their experiments with various insects Schultz and Folsom (18, 19, 20, 21, 22) have been able to transmit these diseases exclusively by aphids, in which case they have, in general, worked with a large number of plants and insects. In their last paper (8) they mention the probability that also other insects may play an important part, but this is not based on exact experiments. That Elze (5) succeeded in transmitting virus diseases by nearly all insects he used, both sucking and others, should also be ascribed in large part to the fact that he infected, in general, a greater number of plants than did Smith. Moreover, he kept the plants growing for a longer period owing to the circumstances under which the experiments were performed, so that the chance of the virus reaching the tubers grew considerably. In several cases also Elze reinfected the plants with virus-bearing insects in the course of the same experiment. To this ensemble of factors may be ascribed his success in transmitting leaf roll and mosaic by means of leaf hoppers, capsid bugs, flea beetles, and caterpillars of *Mamestra brassicae* L. On account of these experiments and his field observations, however, he concludes that all these insects are of hardly any significance for the spreading of virus diseases under normal circumstances. Among the aphids, *Myzus persicae* is by far the most important. Of the other aphids, *Aphis rhamni* B. de Fonsc. (*abbreviata* Patch, see Elze 5, p. 32) and *M. pseudosolani* Theob. transmitted various diseases; with *A. fabae* Scop. only mosaic was transmitted in a few cases.

The particular significance of *Myzus persicae* is explained by Elze from the fact that a definite biological relation between the virus (in this case of leaf roll) and *M. persicae* seems to exist. This is apparent from:

1. The existence of an incubation of the virus in the insect.
2. The retention of the infecting power of the insect after the molt.
3. The preservation of this power after the aphids have lived for about a week on plants not susceptible to leaf roll (spinach).
4. The great certainty with which a small number of individuals can transmit the disease.

These observations were confirmed in 1929 by Smith (26), as was the fact that the virus is not transmitted from the mother insect to the larva.

Lastly, Murphy and McKay (14) published in 1929 the results of their researches, carried on since 1922, on the dissemination of leaf roll by various insects, more especially by aphids. They fully confirm the fact already stated by other investigators that, besides *Myzus persicae*, other insects are of little significance. This fact was doubted by them in former publications. Yet it is remarkable that also with *Macrosiphum solanifolii* Ashm. and *Myzus pseudosolani* so few results were obtained. Especially on *Macrosiphum solanifolii* this is not in accordance with the facts recorded by Schultz and Folsom (19, 20), though Smith (26, 27) also got no positive results from his few experiments.

In order to study the differences of disease-disseminating power of various insects in a more exact way, the following experiment was carried out by the writer in 1928. The method described earlier (5, p. 38) was used. In each of 4 separate but adjoining greenhouses, 22 healthy tubers were planted together with 1 tuber from a leaf-roll plant. The 22 healthy tubers in each greenhouse consisted of 8 tubers of the variety Paul Krüger (President), 7 of Eersteling (Duke of York), and 7 of Roode Star (Red Star). All tubers were planted in separate pots; otherwise, there was no partition between the plants in any one house. In this way repeated infection of the healthy plants by insects from the diseased plant was possible. A path divided each greenhouse into 2 parts. The diseased tuber in each case was cut in half, the halves were planted in separate pots, and the pots were placed one on each side of the path. The healthy plants of the 3 varieties were placed around the diseased one in a regular manner, so that the chance of infection might be equal for each variety. Conditions were essentially the same in each of the 4 greenhouses. For the spreading of the disease in the first house, *Myzus persicae*, in the second, *Aphis rhamni*, in the third, *A. fabae*, and in the fourth, the flea beetle, *Psylliodes affinis* Payk., were used. The strain of *M. persicae* had for some years been raised in the greenhouses on healthy potatoes and on spinach, and that of *A. rhamni* on healthy potatoes and on *Rhamnus cathartica* L. The potatoes on which these strains had been raised during 1928 were still healthy in 1929. *Aphis fabae* was gathered in the field from broadbeans and occasionally from healthy potato plants standing among the beans. In the neighborhood no diseased potatoes were to be found. Notwithstanding these precautions, there was perhaps some danger of the transmission of diseases by *A. fabae* from the field into the houses. This method, however, was adopted because in the environment of Wageningen there is no extensive spread of *A. fabae* to potatoes and multiplication on this host is usually slow. It was expected that by choosing insects which already had passed on to potato and had multiplied there sufficiently, this difficulty would be presented in less degree. The flea beetle, *Psylliodes affinis*, had to be gathered on potato exclusively; this was done on a part of the experimental plots where only healthy potatoes were growing.

As some diseased (leaf roll) tubers of the Green Mountain, Irish Cobbler, and Rural varieties from America were available, these varieties were used in the experiment. This was done in order to determine whether net necrosis, observed in America as a symptom of primary leaf roll, can be produced in Dutch varieties when leaf roll from America serves as a source of infection. For comparison with the leaf roll of American source a diseased tuber of the Dutch variety Bevelander was used. The experiment was arranged so that in the house where *Myzus persicae* was the

vector, the Green Mountain variety was used as a source of disease; in the house where *Aphis rhamni* was the vector the Bevelander was used; in the house with *A. fabae* the Rural was used; and in the house with *Psylliodes affinis* the Irish Cobbler was used.

The results of this experiment as to the appearance of net necrosis are published elsewhere (6). Such a combination of the solution of two problems in one experiment was considered admissible because, from a comparison of the American and the European literature on leaf roll, it probably is safe to conclude that leaf roll is identical on both continents, a fact confirmed by these results.

The results as to the spreading of leaf roll are presented in table 1.

TABLE 1.—*Rate of spread of potato leaf roll in the presence of various insects*

House	Insect	Source of infection	Infested with leaf roll			Total
			Pres.	D. o. York	Red Star	
1	*Myzus persicae*	Gr. Mountain	8	7	7	22
2	*Aphis rhamni*	Bevelander	4	4	5	13
3	*A. fabae*	Rural	3	1	2	6
4	*Psylliodes affinis*	Irish Cobbler	3	2	3	8

Before entering into a more detailed discussion of these results the following comments should be made: The next year's crop of the plants from the first house all showed, besides leaf roll, a disease of the mosaic type; apparently the Green Mountain tubers used had, besides leaf roll, a mosaic disease. Likewise, several plants from the houses with Rural and Irish Cobbler, respectively, showed the next year, besides leaf roll, thin weakened stalks that were strongly suggestive of spindling sprouts. Before the planting, however, the sprouts were quite normal. Though the plants concerned were those on which insects, caught in the field, had been put, it is very improbable that the cause of this malady should have been transmitted by these insects, as such a disease was only sporadically present in the experimental field plots from which they were taken. The disease factor must have been transmitted thus from the leaf-roll plants of the Rural and Irish Cobbler varieties. As it did not appear in either of the two other greenhouses it cannot be a symptom of leaf roll, as Schultz and Folsom (20) originally thought spindling sprout to be. One rather gets the impression of a separate virus disease, as had been surmised before by Elze (5, p. 45).

The tubers of the Duke of York variety were of the same origin as those with which Quanjer and Oortwijn Botjes (17) produced top necrosis by grafting on the variety President. Though with these experiments both varieties were used side by side and there was nothing to interfere with the passage of the insects from a Duke of York plant to a President, the next year's crop of President showed in no case top necrosis. An explanation of this is difficult; possibly the insect did not take the virus of top necrosis so easily, as most of them were infected already with the leaf-roll virus. Possibly the top necrosis is like aucuba mosaic, difficultly transmissible by insects. The last supposition is made probable by the fact that the Duke of York variety is regularly cultivated by the side of other varieties without the occurrence of top necrosis on the latter.

Returning to the discussion of the results concerning the problem why the experiment was started, it appears that in all cases a rather high percentage of the plants had become diseased. This seems to be in contradiction to the English-Irish experiments, so far as it concerns the plants on which *Aphis rhamni, A. fabae,* and *Psylliodes affinis* were brought; this contradiction, however, is not so important as it would seem to be. The circumstances under which the plants were grown in the houses, viz, comparatively high temperature and humidity and scarcity of light, lengthened their growing period. Thus, the chance of infection increased, as well as the possibility that, after the infection, the virus was able to reach the tubers. Already, in the description of the arrangement of the experiment, the possibility of a repeated infestation of the healthy plants was advanced. In the houses with *Myzus persicae* and *Aphis rhamni* these aphids multiplied so fast that, to prevent an early withering of the plants, these houses had to be fumigated with a nicotine preparation. After the fumigation new aphids were brought on to the plants. *Aphis fabae* multiplied less strongly. To contrive, however, a regular dissemination over all the plants and to be sure of a repeated infestation, several aphids were at intervals transmitted from the diseased to the healthy plants. With *Psylliodes affinis* this was more difficult, yet the regular spreading of this insect was managed. These beetles enter the soil for propagation and soon die there. For some months, therefore, beetles just caught were placed 2 or 3 times per week on the diseased plants. The following day the insects still feeding on them were spread over the healthy plants. In this way the possibility of a maximum infection was created in all cases. In spite of this, except in the house with *M. persicae*, a smaller or larger number of plants remained healthy. This difference in attack can be explained only from a real difference in the relation existing between the virus and the various insects used.

In attempting to explain the difficulty with which leaf roll is transmitted by the potato flea beetle, it must be remembered that in insects with

cutting mouth parts the salivary canal usually ends in the pharynx, in which organ the food is mixed with the saliva. With the sucking insects, on the contrary, the sucking canal and the salivary canal are separated from each other as far as the end of the setae. Thus, saliva is first injected into the living plant cell, after which the cell contents are withdrawn by the insect through its sucking canal. With the exception of the probably rare cases of accidental infection through virus sticking to the mouth parts, it must be assumed that with sucking insects the infection of healthy plants takes place through the saliva. The virus is sucked in together with the food and diffuses from the intestinal canal into the blood and from there into the salivary glands to arrive with the saliva back into the plant. It appears from numerous well-studied cases that the infection by sucking insects really takes place in this way. In the first place, the infection occurs with greater certainty than can be explained from the infection with virus residue sticking to the mouth parts. Secondly, it has been shown that the virus is not lost with molting and is preserved if the insects live for some time on plants not susceptible to the disease. See Severin (23), Carsner (2), and others for curly top of sugar beet; Kunkel (11) for aster yellows; McClintock and Smith (12) for spinach blight; Storey (28) for streak of maize; Elze (5) for potato leaf roll; and Ogilvie (15) for a virus disease of lily.

It can be assumed that with insects with cutting mouth parts the virus taken with the food diffuses likewise from the intestinal canal through the blood to the salivary glands. This being true, the virus will come back into the plant very rarely with the taking of food, as the food is not mixed with a virus-bearing saliva before it comes in the pharynx. Consequently, it may be assumed that infection by the potato flea beetle will take place only accidentally through virus residue sticking to the mouth parts.

More difficult is the explanation of the differences in transmitting power of the three species of aphids used. The feeding habits of aphids have been studied by several investigators, and over and over again the observations of Büsgen (1) and Zweigelt (30) have been confirmed, that these insects penetrate with the stylets into the phloem; for *Myzus persicae*, among others, by Smith (24) and for *Aphis fabae* by Davidson (3), while the writer has observed the same for *A. rhamni*. Thus the differences in transmitting power cannot be explained as a mechanical phenomenon. The most plausible concept is undoubtedly the assumption that with the various aphids the leaf-roll virus is attacked to a different degree by the internal organs and their secretions, either in the intestinal duct, in the blood, or in the salivary glands. In other words, there exists a more or less pronounced adaptability of the virus to the various aphids. With *M. persicae* this adaptability is most marked. The researches of Elze (5)

and Smith (27) point to the fact that the virus is able to keep alive for some time in this insect and probably to multiply itself. With *A. rhamni* this adaptability is much weaker, whereas with *A. fabae* the virus seems to be destroyed entirely. Infection seems to take place here exclusively through virus residue that is accidentally present on the stylets. The results of Elze (5) and Murphy and McKay (14) with *M. pseudosolani* and of Schultz and Folsom (20, 21) and Murphy and McKay (14) with *Macrosiphum solanifolii* point to the probability that these aphids, as regards their adaptability are intermediate between *A. rhamni* and *A. fabae*.

If this theory about the connection between the leaf-roll virus and the different potato aphids is right, the following may be concluded:

First. Of each aphid species a certain percentage of the individuals that have lived on diseased potatoes will be able to transmit leaf roll after molting, without sucking any more on the diseased plants.

Second. This percentage of disease transmission by molted aphids will differ only slightly from that by nonmolted ones of the same species. Thus, this percentage will vary from nearly 0 with *Aphis fabae* to about 100 with *Myzus persicae*.

In order to study the correctness of these conclusions, the following experiments were made during the winter of 1929–30. Aphids of the species *Myzus persicae* and *Macrosiphum solanifolii* were reared on leaf-roll tubers. *Aphis rhamni* could not be made use of, for, until now, I have not succeeded in breeding this aphid parthogenetically during the winter, whereas *Aphis fabae* and *Myzus pseudosolani* are growing too poorly on sprouting tubers to make use of them for these experiments.

To be sure that after molting the aphids would not feed any more on the diseased tubers, nearly full-grown larvae were brought from the diseased tubers in little glass tubes and kept therein for a night. In each tube a single individual was kept and its molting observed. The next day the molted and nonmolted aphids were placed each on a separate tuber. The results are shown in table 2.

TABLE 2.—*Retention of the virus of potato leaf roll by aphids after molting*

	Transmission by nonmolted aphids		Transmission by molted aphids	
	Number of tubers		Number of tubers	
	Treated	Diseased	Treated	Diseased
Myzus persicae	11	10	10	7
Macrosiphum solanifolii	34	7	25	9

The number of treated tubers is rather small; still, from the figures, it is evident that *Macrosiphum solanifolii* does not lose the virus during the process of molting, just as has been shown earlier for *Myzus persicae* (5). However, the percentage of the treated tubers that became diseased was much smaller with *Macrosiphum solanifolii* than with *Myzus persicae*, and this was the case when molted as well as when nonmolted aphids were used. So, the results are in accordance with the assumption as to the cause of the differences of the potato aphids in transmitting leaf roll. However, contradictory to the results of Murphy and McKay (14) and Smith (27), the transmitting power of *Macrosiphum solanifolii* seems to be large enough to be significant whenever this aphid is numerous.

According to the researches of Elze (5) and of Smith (25, 26, 27), other sucking insects, such as leaf hoppers and capsid bugs, have no greater significance for the spreading of leaf roll than *Psylliodes affinis*. It is possible, as Smith (24) has shown, that this may partly be ascribed to the fact that these insects, while feeding, do not tap the phloem so regularly as do aphids. Relative to researches on such diseases as curly top, aster yellows, etc., where leaf hoppers are the only spreaders of the diseases, it does not seem probable that this circumstance is very important. It is rather to be assumed also that with these insects the virus, after reaching the intestinal duct, is for the greater part destroyed. It should also be taken into account that in many cases the plant cells react so violently to the sucking of these insects that the protoplasm soon dies. For the further spreading of the strongly parasitic virus through the plant this should be an unfavorable circumstance, as already Smith (24) has asserted.

In relation to this it should be noted here that, according to the description of Wille (29), the capsid bug *Piesma quadrata* Fieb., causing the "Kräuselkrankheit" of the beet in Germany, does not cause such a violent reaction as appears from the early symptoms of this disease. Wille states that the Kräuselkrankheit should be included among the virus diseases. Also, the leaf hoppers *Eutettix tenellus* Bak., *Cicadula sexnotata* Fall., and *Balclutha mbila* Naude apparently do not cause necrotic spots.

In America necrotic spots develop to a high degree through *Empoasca mali* Le Baron living on numerous plants, but, as far as known, this insect plays no part as a spreader of a virus disease. At least, nothing indicates that the spots observed by Eyer (7) and Granovsky (9, 10) are related to virus diseases.

Elze (5) has given a classification of the virus diseases based on the relation between the virus and the insect with each of these diseases. From subsequent publications it appears that nearly always, when studied more thoroughly, a very close biological relation exists between the virus and at

least one of the insects able to transmit it. This points strongly to the living nature of the virus.

The only exception hitherto known is cucumber mosaic, where, according to Doolittle and Walker (4), *Aphis gossipii* Glov., as well as the beetles *Diabrotica vittata* Fabr. and *D. duodecimpunctata* Oliv., loses its transmitting power soon after leaving the diseased plant, so that in this case infection takes place only mechanically. In the remaining cases a distinction can be made between diseases (such as curly top and aster yellows) that can be transmitted by but one insect species and diseases that can be transmitted by a greater number of insects. In the latter only one or a few of these insects stand in closer biological relation to the virus. The remaining insects transmit the disease only mechanically and are mostly of little importance. Spinach blight and leaf roll can be placed in this group. It may prove necessary to separate aster yellows from those diseases that are transmitted by one insect species because of the long incubation period (10 days) of the virus in the insect. In all other cases, where the incubation period is determined, it lasts only 1 or 2 days.

There yet remains a group of diseases in which the transmission by insects has not yet been successful. Among such are peach yellows, sandal spike, and abutilon mosaic.

SUMMARY

The present-day knowledge of the significance of various insects in relation to the spread of the virus diseases of the potato is discussed. *Myzus persicae*, which is particularly well adapted for spreading such diseases, has been studied especially in connection with leaf roll.

There are different opinions as to the ability of other insects to transmit the viruses of the potato. It is suggested that such differences are due to the use of different experimental methods. European research workers generally are of opinion that *Myzus persicae* is the only insect that plays an important part.

Experiments are described on the spread of leaf roll from the varieties Bevelander, Green Mountain, Rural, and Irish Cobbler to the varieties President, Duke of York, and Red Star by the aphids, *Aphis rhamni*, *Myzus persicae*, and *A. fabae*, and the flea beetle, *Psylliodes affinis*. In these experiments the most favorable circumstances for infection were provided. The table of results shows that *M. persicae* infected all 22 plants, *A. rhamni* 13, *A. fabae* 6, and *P. affinis* 8 plants.

The small transmitting power of *Psylliodes affinis* is ascribed to the fact that insects with cutting mouth parts can cause infection only through virus residue that may accidentally adhere to the mouth parts. With the aphids and other Hemiptera, on the contrary, the differences are explained

by the assumption that the virus taken with the food is more or less destroyed by the organs of the insect, according to the degree of adaptability of the virus to the insect.

The correctness of this assumption is made probable by an experiment, wherein it is shown that *Myzus persicae* and *Macrosiphum solanifolii* do not lose their transmitting power when molting and that the percentage of virus-bearing individuals that are able to transmit the disease is nearly the same before and after the molt. Under the same conditions the transmitting power of *Myzus persicae* was much greater than that of *Macrosiphum solanifolii* (Table 2).

Considering these results and those of other investigators, the following classification is given, based on the relation of virus diseases to insects.

1. Viroses which, according to our present knowledge, are not spread by insects, e.g., peach yellows, sandal spike.

2. Viroses which are spread by different insects and are not specially adapted to any of these insects, e.g., cucumber mosaic.

3. Viroses which are adapted to particular insects.

   A. Which, in addition, are transmitted mechanically by other insects, e.g., potato leaf roll, spinach blight.

   B. Which are transmitted only by the insect to which the disease is adapted.

      a. With a short incubation period of the virus in the insect, e.g., curly top, streak of maize.

      b. With an incubation period of about 10 to 14 days, e. g., aster yellows.

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THE RELATION OF PHYSIOLOGIC SPECIALIZATION IN TILLETIA TO RECENT EPIPHYTOTICS OF BUNT IN DURUM AND MARQUIS WHEATS[1]

C. S. HOLTON[2]

INTRODUCTION

In a previous publication[3] the writer pointed out that, prior to 1925, bunt caused virtually no damage to durum wheats in the hard-red-spring-wheat region of the United States but that its prevalence and destructiveness have increased so greatly since that time that the disease has assumed major importance. Evidence was presented that this increase in the severity of bunt in durum was due to the existence of one physiologic form, or possibly several, of *Tilletia tritici* (Bjerk.) Wint., apparently not present or, at least, not widely distributed previously. The present paper reports the results of experiments that substantiate this conclusion. In addition, it has been found that Marquis, previously highly resistant in the field in the spring-wheat region, became severely smutted in many fields in 1930. The results of inoculations indicate clearly that this increased amount of bunt in Marquis apparently is due also to the existence of at least one hitherto unknown form of *T. levis* Kühn. Marquillo, a newly developed stem-rust-resistant common wheat also seems susceptible to the new form. Gaines[4] has reported similar experiences with certain bunt-resistant varieties of wheat in Washington.

MATERIALS AND METHODS

Ten varieties of common and durum wheats and of emmer were inoculated with four collections of *Tilletia tritici* and two collections of *T. levis* in the field at University Farm, St. Paul, Minnesota, in 1930 (Table 1). The seed was treated with formaldehyde (dip method), washed in water, and, when dry, inoculated at the rate of 0.5 gram of spores to 100 grams of seed. After inoculation, the seed was sown in triplicated, systematically-distributed, 8-foot rows in the field.

[1] Published with the approval of the Director of the Minnesota Agricultural Experiment Station as paper No. 978 of the Journal Series of the Minnesota Agricultural Experiment Station.

[2] The writer is greatly indebted to Dr. E. C. Stakman for invaluable suggestions offered in the writing of this paper.

[3] Holton, C. S. A probable explanation of recent epidemics of bunt in durum wheat. Phytopath. **20**: 353–357. 1930.

[4] Gaines, E. F. New physiologic forms of *Tilletia levis* and *Tilletia tritici*. Phytopath. **18**: 579–588. 1928.

TABLE 1.—*The percentages of smutted heads in ten varieties of wheat and emmer inoculated artificially with four collections of Tilletia tritici and two collections of T. levis at University Farm, St. Paul, Minnesota, in 1930*

Class, variety and C. I. number	Inoculum, source of inoculum, and percentage of smutted heads					
	Tilletia tritici				Tilletia levis	
	California	Manitoba, Canada	Devils Lake, North Dakota	Litchfield, Minnesota	Ivanhoe, Minnesota	St. Paul, Minnesota
Common						
Kota 5878	50.8	39.3	36.1	16.1	67.8	61.0
Ceres 6900	20.0	25.6	17.8	9.6	48.1	42.5
Preston 3081	3.1	4.0	3.3	0.3	8.8	5.8
Marquis 3641	2.8	3.6	0.0	0.0	23.3	9.0
Marquillo 6887	2.1	4.5	0.6	0.0	18.5	7.8
Ruby 6047	0.5	1.3	0.0	0.0	2.0	2.5
Hope 8178	0.5	0.0	0.0	0.0	0.0	0.0
Durum						
Mindum 5296	0.3	0.1	30.2	0.7	4.2	2.2
Pentad 3322	3.3	2.6	40.1	2.0	6.3	4.6
Emmer						
Vernal 3686	2.6	0.0	12.3	16.1	4.0	1.8

The percentages of infection (Table 1) are based on counts of 600 heads in all varieties except Mindum, in which case the percentages are based on the total number of heads, ranging from 449 to 600, in 3 8-foot rows. Except for Kota and Ceres, in which smutted heads were easily detected by external examination, smutted heads were determined by clipping each head three times. No distinction was made between partially and completely smutted heads.

A number of commercial wheat fields[5] in various sections (south, west, north) in Minnesota were examined for bunt infection. The percentages of bunt were computed from counts of bunted heads in 10 separate yard lengths of the drill rows, selected at random. Random samples were examined microscopically to determine the relative prevalence of the two species of bunt in the various fields. (Table 2).

RESULTS

The results obtained in the inoculation tests with four collections of *Tilletia tritici* confirm those which have been published—Holton, *l. c.* The California and Manitoba collections probably consist of identical forms, for the virulence of one was very similar to that of the other on the varieties tested (Table 1). Both collections were extremely virulent for Kota, the California collection producing 50.8 per cent and the Manitoba collection 39.3 per cent. In 1929 these collections produced 53.8 and 42.6 per cent of bunt, respectively, on Kota. All other varieties tested, except Ceres, appear to be highly resistant to these collections. The North Dakota collection was most virulent for the durum wheats, Mindum and Pentad, producing in these varieties 30.2 and 40.1 per cent of bunt, respectively, in 1930, as compared with 50.6 and 40.1 per cent, respectively, in 1929. All other varieties tested, except Kota, have been somewhat resistant to this form. The results obtained from this collection in 1930 established the validity of previous conclusions that the apparently increased susceptibility of durum wheats to bunt is due to the presence of a physiologic form of the pathogene previously not prevalent in durum-growing regions.[6] Field observations during the season of 1930 also confirm the conclusion that durum wheats, as a class, are no longer resistant to bunt, as evidenced by the fact that the amount of bunt in commercial fields of Mindum ranged from a trace to 51 per cent (Table 2).

The Litchfield, Minnesota, collection of *Tilletia tritici* produced 23.6 per cent of bunt in 1929 and 16.1 per cent in 1930 on Vernal, while all

[5] Data on commercial wheat fields were obtained by the writer during a survey conducted by the Agricultural Extension Division of the University of Minnesota in cooperation with the Plant Disease Survey, United States Department of Agriculture.

[6] Holton, *l. c.*

TABLE 2.—*Smut produced by Tilletia levis and T. tritici in 31 commercial fields of Marquis and Mindum wheat and Vernal emmer in three widely separated counties in Minnesota 1930*

Variety of grain	Species of Tilletia	Percentage of smut
Marquis	levis	40
"	"	40
"	"	35
"	"	38
"	"	35
"	"	5
"	"	2
"	"	4
"	"	5
"	"	3
"	"	4
"	"	4
"	"	3
"	"	4
"	"	4
"	"	14
"	"	2
"	"	5
Mindum	tritici	40
"	"	51
"	"	5
"	"	8
"	"	5
"	"	4
"	"	7
"	"	5
"	"	11
"	"	tr.
"	"	3
"	"	tr.
Vernal	"	40

other varieties inoculated, except Kota, were highly resistant. In the one field of Vernal observed in 1930, 40 per cent of the heads were smutted with *T. tritici* (Table 2). It is evident that additional experimental data and field observations support the contention that Vernal is no longer resistant to bunt.

Marquis, a common wheat previously markedly resistant to bunt, is now known to be quite susceptible. In 1929 a field of Marquis near Ivanhoe, Minnesota, was reported heavily infected with bunt, approximately 40 to 50 per cent. A sample of bunt was collected from this field, identified as

Tilletia levis and used in the inoculation experiment in 1930. This collection produced 23.3 per cent of bunt on Marquis and proved extremely virulent on Kota and Ceres and moderately virulent on Marquillo (Table 1). When commercial fields of Marquis were examined in 1930 it was not uncommon to find very much higher percentages of bunt than had been reported prior to 1929. In 5 of the 18 fields examined there was 35 per cent or more of bunt (Table 2). It is obvious that Marquis can no longer be considered highly resistant to all forms of bunt, and it is probable that the increased amount of bunt in it is due to a hitherto unknown physiologic form of *T. levis* that, until recently, has not been prevalent in regions where Marquis wheat is grown.

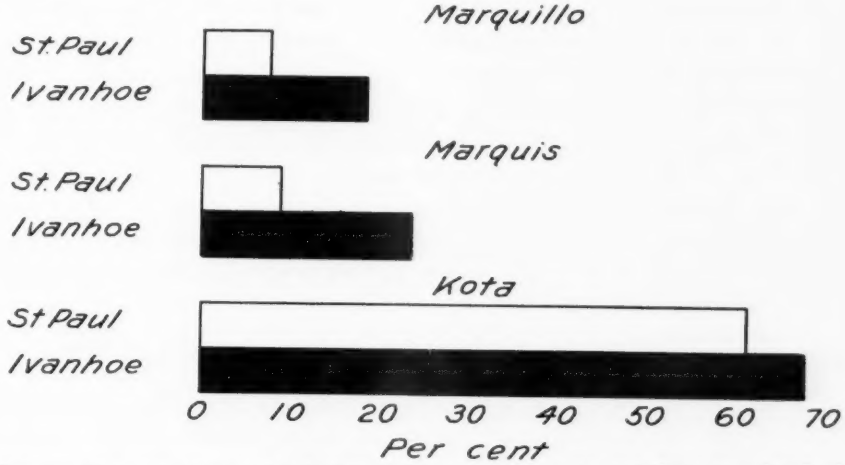

FIG. 1. The percentages of infection in Marquillo, Marquis, and Kota inoculated with spores of two collections of *Tilletia levis* from Minnesota.

It is obvious from the results summarized in table 1 that there are two distinct physiologic forms[7] of *Tilletia levis*, based on their pathogenicity for the varieties inoculated. The form from St. Paul, Minnesota, is extremely virulent on Kota and Ceres, while Marquis and the other varieties tested were either highly resistant or virtually immune. This is in accordance with the results obtained by Rodenhiser and Stakman.[8] The form from Ivanhoe, Minnesota, also is extremely virulent on Kota and Ceres and, in addition, attacks Marquis and Marquillo heavily (see Table 1 and Figure 1). Hence, it is considered distinct from the St. Paul form because of its greater virulence on Marquis and Marquillo.

[7] The propriety of designating these collections as physiologic forms is discussed on page 354 of the writer's first paper (*l. c.*).

[8] Rodenhiser, H. A., and E. C. Stakman. Physiologic specialization in *Tilletia levis* and *Tilletia tritici*. Phytopath. **17**: 247–253. 1927.

Field observations, which are summarized in table 2, indicate that all the fields of Marquis examined were infected with *Tilletia levis*, while all the fields of Mindum, as well as the single field of Vernal, were infected with *T. tritici*.

This observation is borne out by experimental evidence inasmuch as the results in table 1 show clearly that the highest percentages of smut on Marquis and Marquillo are produced by *Tilletia levis*, while the highest percentages of smut on Mindum and Vernal are produced by *T. tritici*. Similar evidence has been presented by Brentzel and Smith.[9]

It is perfectly evident that new physiologic forms of *Tilletia levis* and *T. tritici* have been and apparently still are making their appearance in the hard-red-spring-wheat region. These forms are quite distinct from each other in pathogenic capabilities. Whether these forms were introduced into the region from other regions, whether they arose as a result of mutations, or whether they arose as a result of hybridization between existing forms is not known. It has been shown by Stakman, Christensen, Eide, and Peturson[10] that *Ustilago zeae* (Beckm.) Ung. mutates abundantly and that some of the mutants differ sharply from their parents in pathogenicity. It was shown also by these authors that hybridization is extremely common within the species. There is no reason whatever why new forms of *T. levis* and *T. tritici* should not originate through these perfectly natural processes. It is possible that they may originate as a result of hybridization between different physiologic forms or possibly even between the two species. It also is entirely possible, of course, that they may have been introduced from other regions. Whatever the mode of origin, it is perfectly apparent that new physiologic forms of the two bunt species do appear and that varieties which have been resistant in the field hitherto may be very susceptible to new forms. The practical importance of this fact is perfectly obvious and is very clearly apparent in connection with the bunt situation. For many years, the durums remained almost free from bunt in the upper Mississippi Valley and Great Plains region. It was shown, as a result of extensive inoculations, that virtually all of the durum varieties actually were highly resistant to those forms of the bunt species which seem to be most prevalent, but the durums can no longer be considered resistant for practical purposes, as they are very susceptible to certain physiologic forms that are widely enough distributed to cause real epiphytoties of bunt in durum wheats. Furthermore, Marquis, extensively grown in the spring-wheat region since about 1915, has also been highly resistant to bunt in the field. It was also

[9] Brentzel, W. E., and Ralph W. Smith. Varietal resistance of spring wheats to bunt. N. Dak. Agr. Exp. Sta. Bul. 231. 1929.

[10] Stakman, E. C., J. J. Christensen, C. J. Eide, and Bjorn Peturson. Mutation and hybridization in *Ustilago zeae*. Minn. Agr. Exp. Sta. Tech. Bul. 65. 1929.

decidedly resistant to all collections of bunt with which it was inoculated artificially in the experimental plots at University Farm, St. Paul, Minnesota, for at least 10 years. During the summer of 1930, however, the writer showed that this variety is very susceptible to at least one physiologic form of *T. levis*, which also has become widely enough distributed geographically to cause considerable losses to Marquis in the field. Marquillo, which because of its parentage would be expected to be highly resistant and which actually has been resistant when grown in the field, also is susceptible to at least one physiologic form of *T. levis*. From previous experience it seems likely that this variety also may become rather heavily bunted after it has been grown commercially for a sufficiently long time to permit the accumulation of inoculum on the seed. It seems very clear, therefore, that the bunt situation is continually changing and that varieties, resistant in one locality, may be susceptible in another. Furthermore, varieties may be resistant for a number of years in the same locality only to succumb to attacks of bunt when new physiologic forms make their appearance. The exact nature of resistance of varieties of wheat to bunt is not clearly understood. It seems probable, however, that varieties in order to be long useful from the standpoint of bunt resistance may have to have some sort of structural or functional resistance that will enable them either to resist or escape the attacks of the pathogene. It seems highly desirable, therefore, to make basic studies on the nature of resistance. In the meantime the writer considers it of the utmost importance to continue investigations toward the perfecting and simplification of seed treatments. It would seem extremely important to continue very vigorously the campaign for the treatment of all seed wheat and particularly of new and improved varieties. One can never be sure of the bunt situation at any given time, and it would seem to be the part of wisdom, therefore, to use to the fullest possible extent the method of bunt control which is known to be efficacious, namely, seed treatment, and, in some regions, crop rotation.

SUMMARY

1. The virulence of four collections of *Tilletia tritici* and two collections of *T. levis* was compared on ten varieties of Triticum.

2. The results with *Tilletia tritici* confirmed those already published[11] Obviously, the recent epiphytoties of bunt in durum wheats are caused by a physiologic form which has not been prevalent until fairly recent years. Experimental evidence and field observations in 1930 also confirm the conclusion drawn from results obtained in 1929 that Vernal, previously resistant, now is susceptible to bunt, because of the appearance of a form of *T. tritici* previously unknown.

[11] Holton, *l. c.*

3. Two physiologic forms of *Tilletia levis* were identified on the basis of their reaction on Kota, Ceres, Marquis, and Marquillo. The St. Paul, Minnesota, collection represents one form which is distinguishable by its high degree of virulence on Kota and Ceres and low virulence on Marquis and Marquillo. The collection from Ivanhoe, Minnesota, represents another form which attacks Marquis and Marquillo, as well as Kota and Ceres, with a high degree of virulence.

4. Field observations have confirmed experimental evidence that recent outbreaks of bunt in Marquis wheat are due to the presence of a hitherto undescribed physiologic form of *Tilletia levis*.

5. Under field conditions *Tilletia levis* is the predominant species occurring on Marquis, and *T. tritici* is the predominant species on Mindum and Vernal. This observation is confirmed by experimental results which show that Marquis is quite susceptible to *T. levis* and highly resistant to *T. tritici* while the reverse is true with Mindum and Vernal.

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THE EFFECT OF ULTRA-VIOLET LIGHT RADIATIONS ON THE VEGETATIVE GROWTH OF WHEAT SEEDLINGS AND THEIR INFECTION BY ERYSIPHE GRAMINIS[1]

G. L. Hey and J. E. Carter

EXPERIMENTAL PROCEDURE

Source of light: The lamp used was a quartz mercury-vapor lamp (Hanovia Artificial Alpine Sun, 2.5 amps., 210 volts direct current type). An interval of 3 minutes was allowed to elapse after lighting up before using the lamp, in order to allow it to become steady. At the intensities employed there was very little heat effect, as registered with an ordinary mercury thermometer.

The objects to be irradiated were grouped directly under the light, the outer pots or boxes being tilted slightly to allow the light to fall normally on the leaves. Distances were measured from the center of the quartz tube to the tips of the plants.

Source of infection: The plants were kept in a greenhouse where 100 per cent infection had occurred in susceptible varieties for several years past and where non-treated wheat plants were already showing such infection.

Material used: The varieties of wheat used were Squarehead's Master, Little Joss, American Club (susceptible), and Persian Black (immune).

PRELIMINARY EXPERIMENTS

A few preliminary experiments were conducted in order to determine the quantity of light that would be effective in reducing the infection of the plants by the mildew without greatly damaging the plants themselves; and also to determine whether irradiation with ultra-violet light would render the immune variety, Persian Black, susceptible to infection. Of these experiments the most conclusive was as follows:

Seeds of Persian Black, Little Joss, and Squarehead's Master were sown in earth and 24 days later were placed in 4½-inch pots, 4 plants per pot. After an interval of 2 days, to allow the plants to become established, 2 pots of each variety were irradiated daily for 3, 10, and 15 minutes, respectively, at 24 inches from the light, and 2 pots of each variety were left

[1] This work was carried out at the suggestion of Dr. W. A. R. Dillon Weston, as a continuation of his own work on the action of ultra-violet light on some specific fungi.[2] To him the writers wish to express their gratitude for the suggestion and for the loan of apparatus and material; also to E. T. Halnan, Esq., for his advice and assistance.

[2] Weston, W. A. R. Dillon. The fungicidal action of ultra-violet radiation. Phytopath. 20: 959–965. 1930.

non-treated as controls. The treatment was continued 14 days, and the plants were examined within 24 hours after the last irradiation and again a week later. Before irradiation commenced, with the exception of the Persian Black seedlings, all the plants were badly infected with mildew.

First examination: Persian Black. All plants perfectly free from mildew. Those treated 3 minutes no scorching effect, 10 and 15 minutes badly scorched.

Little Joss and Squarehead's Master. Controls, all badly mildewed, every leaf attacked. Treated 3 minutes, mildew slight and only on old, lower, yellowed leaves on surface away from light; new leaves free. Treated 10 and 15 minutes, almost no mildew, leaves badly scorched. In every case Squarehead's Master was more highly infected than Little Joss.

Second examination: Persian Black still noninfected. Squarehead's Master and Little Joss plants all badly infected.

This experiment shows that by a suitable irradiation it is possible to render infected plants almost free from disease, but, at the concentrations so far employed, the plants themselves were severely injured. It was therefore determined to discover if, commencing with plants free from mildew, suitable irradiation would keep them clean without deleterious effect on their growth.

SUBSEQUENT EXPERIMENTS: EXPERIMENT NO. 1

Little Joss and Persian Black: Pots of Little Joss and Persian Black seedlings, about 11 cm. high and free from mildew, were treated 5 days as follows:

1, 5, 10	minutes	once	a day	at	24	inches	from	the	light.
1 and 5	"	twice	"	"	"	24	"	"	"	"
1	minute	once		"	"	"	48	"	"	"	"
1	"	twice		"	"	"	48	"	"	"	"

There were 2 pots of each of these treatments of both varieties, and, in addition, 3 pots of each variety were left as controls.

The plants were examined the day after the last irradiation and again a fortnight later.

First examination: Persian Black. All plants free from mildew. No scorched plants except in those cultures treated 5 and 10 minutes.

Little Joss. Controls, all plants badly infected with mildew: no scorch.

Treated.

1	minute	at	48	inches	once.	Mildew just showing. No scorching.
1	"	"	48	"	twice.	No mildew. No scorching.
1	"	"	24	"	once.	Mildew very slight. No scorching.
1	"	"	24	"	twice.	No mildew. No scorching.

5	"	"	24	"	once.	"	"	Slight scorching.
5	"	"	24	"	twice.	"	"	Badly scorched.
10	"	"	24	"	once.	"	"	Slight scorching.

Second examination: Persian Black. All plants still free from mildew. It was very noticeable that although all the plants were exactly alike at the commencement of the experiment, those irradiated 1 minute a day at 48 inches were all much stronger and taller than any of the others.

Little Joss. All plants badly mildewed; the controls, nearly killed. No noticeable difference in height between any of these plants, as in the case of Persian Black.

SUMMARY

The general result of this experiment seems to show that it is possible to render the plants free from mildew without causing any visible harmful effects on the plants themselves. When irradiation was discontinued the plants became infected. The effect of irradiation twice a day seems greater than irradiation for the same total time once a day.

In order to test the results of Experiment No. 1 more accurately, the following experiment was devised.

EXPERIMENT NO. 2

Three boxes of wheat seedlings, approximately 8 cm. high and about 300 plants per box, one box, each, of American Club, Little Joss, and Persian Black, were each divided into three approximately equal portions. One of these divisions was left untreated as a control, and the other two were irradiated, the one for 1 minute and the other for 3 minutes a day for 15 days, at 48 inches from the lamp. At the commencement of the experiment the seedlings were all free from mildew.

Twelve hours after the last irradiation most of the seedlings were taken up and an average sample of 100 from each division in each box was carefully measured for height and examined for mildew. Those plants upon which not a single pustule of the fungus could be seen by the naked eye were recorded as clean.

TABLE 1.—*Average height, in centimeters, of the seedlings of American Club, Little Joss, and Persian Black treated 1 minute and 3 minutes with ultra-violet light*

Variety	Control	Treated 1 minute	Treated 3 minutes
American Club	23.5	24.8	22.1
Little Joss	24.1	27.5	23.5
Persian Black	21.8	21.9	21.4

The Persian Black seedlings became "lodged" so that the light was not equally distributed on all the plants in each section, and therefore the re-

sults cannot be quite comparable with those obtained on the other two varieties.

TABLE 2.—*Percentage of American Club, Little Joss, and Persian Black wheat plants infected by mildew following exposure to ultra-violet light for periods of 1 and 3 minutes, respectively*

Variety	Number clean	Number infected	Percentage infected
American Club			
Control	22	78	78
1 minute	26	74	74
3 minutes	90	10	10
Little Joss			
Control	0	100	100
1 minute	9	91	91
3 minutes	48	52	52
Persian Black			
All clean			

Those seedlings left in the boxes were kept without further treatment for a week and then examined, when it was seen that the Persian Black plants were still not infected, while those of the other two varieties were all badly attacked by mildew.

SUMMARY

With American Club and Little Joss 1 minute of irradiation gave increased growth but only slightly reduced the percentage of infection; while irradiation for 3 minutes reduced the growth but greatly reduced the percentage of plants infected by mildew; but when irradiation ceased the plants again became infected.

In the case of Persian Black the effect on growth was not so marked, and its immunity from mildew was not affected.

DISCUSSION

In these experiments it was found possible to give the plants an amount of ultra-violet light irradiation which gave fairly effective control of the fungus without causing much damage to the host.

The light apparently acts directly on the fungus mycelium, killing it or rendering it dormant. The action is not an indirect one affecting the metabolism of the host in such a way as to render it immune or resistant. This is shown by the fact that the fungus was suppressed only on the side of the leaves exposed to the light. (In some of the experiments the pots were turned about so as to expose all surfaces to the light, and in these cases the fungus was killed on both sides of the leaves.) Also, when irradiation was

stopped the plants became as readily and severely infected as before. Since, however, certain quantities of light were found to give increased growth, this would have an indirect effect in enabling the plants to flourish in spite of slight infection.

Smaller quantities of light were required to keep clean plants free from disease than to kill the fungus on infected plants. This probably means that the young hyphae are more susceptible to ultra-violet irradiation than are the older mycelia.

It was found impossible, even when using quantities of light which produced severe scorching, to break down the resistance to infection of the variety Persian Black. This is contrary to what might have been expected, since cutting or burning leaves of cereals will often break down their resistance to attacks of mildew.[3]

In view of the fact that certain quantities of irradiation gave increased growth of the wheat plants (which confirms a result obtained by Delf, Ritson, and Westbrook),[4] it would have been interesting to correlate the quantity of ultra-violet-light irradiation to which the plants were subjected with the quantity occurring in normal summer sunshine. An attempt was made to do this, but it was found impossible. It is a well-known fact, however, that in England the hotter and drier the summer, the greater the yield of wheat. This is borne out by the following figures: In 1929 the wheat yield averaged 19.1 cwt. [31.8 bushels] per acre and the weather conditions were exceptionally hot and dry and the incidence of mildew was slight.[5] This year the weather has been wet and the incidence of mildew fairly great; the yield is estimated at only 15.8 cwt. [26.3 bushels] per acre. The 10 years' average is 17.7 cwt. [29.5 bushels]. It is probable that in an English summer the amount of ultra-violet irradiation is never sufficiently high to give the maximum yield of which the wheat crop is capable. But, the amount may be sufficient, as in 1929, to suppress the growth of the fungus, and this is possibly one of the factors influencing the yield; whereas, in 1930, the amount was so low that the fungus flourished and the crop thereby suffered.

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TURGESCENCE AND RUPTURE OF POTATO TUBER

H. G. MacMillan

A condition arose recently in a field of potatoes under irrigation which gave opportunity for a limited study of some effects of high turgescence in tubers. The potatoes were of the Bliss Triumph variety. They were of excellent quality and the yield was satisfactory, as 320 bushels per acre of marketable tubers were harvested. However, nearly half of the tubers showed an unusual condition of injury.

The injury consisted of newly made cracks, fairly deep into the tuber flesh and of unequal and indeterminate extent, often shaping into curious and fantastic fissures. There was no regular design in the rupture. The usual depth was $\frac{1}{4}$ to $\frac{3}{8}$ of an inch below the surface. Some tubers showed a single crack an inch or more in length and quite straight; some were curved on the surface immediately presented to view; while others followed around the tuber, virtually ringing it, as though the tuber were about to break in two pieces. The majority were unequally radial, the cracks extending out from a central point in distorted star shapes. There were still other designs. When freshly made, the openings were white and gleaming within but soon became gray, as the surface dried and shrank. The injury was being caused in digging. The tubers in the ground were clean, bright, and free from cracks. The process of digging bumped them over the elevator of the digger, against each other, and finally out behind with a fall of a foot or more to the ground. The fall from the digger where one tuber struck another already on the ground was enough to cause the bursting and rupture of one or both of them. The soil was a moist sandy loam, free from stones and clods, or the damage would have been greater. The process of picking, sorting, and sacking, all of which went on in the field soon after digging, added further damage.

The condition of high turgidity and the resisting tension which the tuber offered could not be measured but could be illustrated. Upon pricking the surface with a sharply pointed knife to the depth of $\frac{1}{4}$ inch the fissures would form instantly, some for a length of 2 inches or more. Tubers were found which, on being once quickly stabbed with a pin, would break out a star-shape rupture with radii as much as an inch long. Yet no tubers could be found that, on being carefully taken from the ground, showed any trace of rupture or indication of this tendency.

A number of tubers were dug, taken to the laboratory in a closed vessel, brushed carefully to remove soil particles, and weighed. They were laid on a table freely exposed to the air. At 2-day intervals they were

reweighed and two or more subjected to pricking with a knife or dropped a distance of 12 inches to the table. There was a gradual decline in weight and a corresponding diminution in the extent of the rupture. It was necessary to discard ruptured tubers. At the end of 8 days no rupturing occurred except on application of unusual and unwarranted violence. The few remaining tubers, which appeared as sound and fresh as when dug, were weighed. The weights are shown in table 1.

TABLE 1.—*The weights of 8 tubers as dug in the field, the weights after air-drying for 8 days, the difference in grams, the percentage loss in weight for each tuber, and averages*

Tuber no.	Weight as dug	Weight after 8 days air drying	Weight loss	Weight loss
	Grams	*Grams*	*Grams*	*Per cent*
1	521.65	484.70	36.95	7.083
2	234.81	213.41	21.40	9.113
3	231.95	216.42	15.53	6.695
4	216.30	208.37	7.93	3.666
5	186.32	181.21	5.11	2.742
6	170.70	158.90	11.80	6.912
7	119.22	114.02	5.20	4.361
8	63.29	60.00	3.29	5.198
Average	218.03	204.63	13.40	6.145

The day after digging began and 2 days prior to the call to the field, a 0.30 inch rain fell. Following the rain a moderate frost killed the tops of the vines. The large amount of water available to and taken up by the uninjured roots evidently was forced into the tubers. That they were unequally filled with water of turgescence was indicated by the varying damage in the field and by the unequal shrinkage of the tubers weighed and dried to the nonbursting point. The heavier tuber, weighing a pound or more, would be liable to greater damage than the smaller one falling the same distance. The area of impact would be about the same in each case, but the falling weight behind it put a greater strain on the contact area of the larger potato. The larger tubers showed more damage than the smaller. The table shows that of the few tubers reaching a final weight it was necessary for them to lose an average of 6.14 per cent of their weight as dug to be restored to an approximately normal condition. If the dried weight is regarded as the normal weight it would appear that an increase of approximately 6.5 per cent in weight due to excess water is possible without damage to the potato, except as the result of rough handling. Careful selection of individual tubers would materially raise this amount,

as would be indicated from the weight of tuber No. 2. With the decline and death of the vines no doubt the tubers would have soon lost water to the point where they could have been dug with safety. Postponed digging would have controlled the trouble.

The freshly exposed ruptured tissue began at once to dry and to form a covering of dried and collapsed cells. The open area exposed to air was $\frac{1}{4}$ to $\frac{3}{8}$ of an inch deep. Callous tissue, however, formed more deeply, extending $\frac{1}{2}$ inch or more below the surface. This indicated that the deeper-lying tissues were wrenched and torn, though they were not visibly spread apart. The tissues at the surface of the tuber, probably inclusive between the epidermis and the vascular layer, seemed to restrain the rupture. The pressure appeared to exist only in the pith or below the vascular region. Cutting the tubers revealed no water-soaked areas within. All tissues were normal in appearance.

No additional cases of injury equal to the one described have been observed. Small ruptures have been observed many times in irrigated potatoes, where the cracks have been $\frac{1}{2}$ inch long or less and shallow. The usual result of these ruptures is the development of small pads of corky or callous tissue within the flesh. The former are soon lost to view. In the case described there was no opportunity for extended study. The potatoes were soon dug and moved. To attempt to duplicate the conditions experimentally would have been difficult, if not impossible, and, in view of the restricted local damage, entirely unwarranted.

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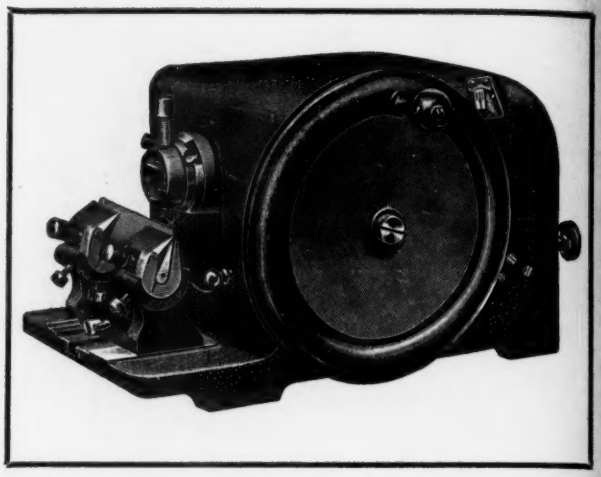

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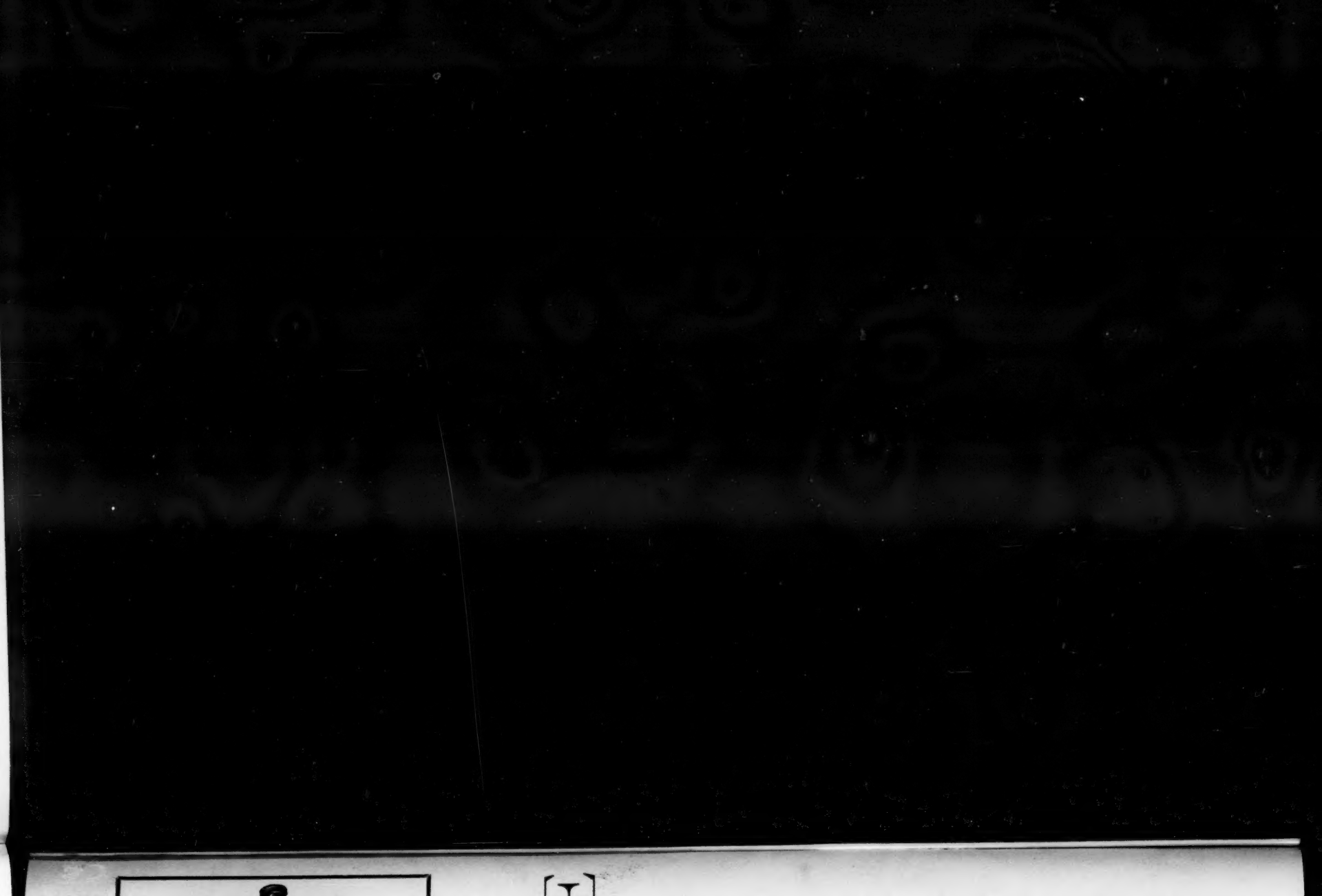
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